44th Summer Medical Student Research Day

ABSTRACTS

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ABSTRACT

RANDOMIZED TRIAL COMPARING ULTRASOUND GUIDED AND LAPAROSCOPIC TRANSVERSUS ABDOMINIS PLANE BLOCKS: A PILOT STUDY. Arslan Arshad. Sponsored by Paul F. Rider, MD, Department of Colon and Rectal Surgery, University of South Alabama Medical Center, Mobile, AL.

Effective analgesia is an essential part of postoperative management. Sufficient pain control enhances early recovery, increases patient satisfaction, enables early mobilization, and prevents postoperative complications such as deep vein thrombosis, pulmonary embolism, pulmonary atelectasis, pneumonia, delayed wound healing, and constipation. Optimum pain control can thus shorten hospital stays, conserve resources, and reduce morbidity or mortality. The transversus abdominis plane (TAP) block is a relatively new regional anesthetic targeting the neural afferents in the neurovascular plane between the internal oblique and transversus abdominis muscles allowing for access to the subcostal, ilioinguinal, iliohypogastric, and T6 to L2 nerves. TAP blocks can be performed under ultrasound or laparoscopic guidance and have been shown to effectively reduce opioid consumption and pain scores after laparoscopic abdominal surgery. While the TAP block is emerging as a standard of care practice, to date no published trials have compared techniques for administering the block. The goal of this study was to compare the efficacy and efficiency of ultrasound guided and laparoscopic TAP blocks. Patients undergoing laparoscopic hernia repair or colorectal surgery were randomly assigned to receive a four-point bilateral ultrasound guided or laparoscopic TAP block (4 x 10mL) comprised of a 2:3 mixture of 1% lidocaine and 0.5% bupivacaine + epinephrine (1:200000), respectively. The primary endpoints were cumulative opioid use at 8, 12, and 24 hours postoperatively and procedural time. No significant difference in postoperative opioid use at 8, 12, or 24 hours was observed between patients who received an ultrasound guided (n=3) or laparoscopic TAP block (n=5). On average, ultrasound guided TAP blocks (7.39±3.72 min) took longer than laparoscopic blocks (3.44±0.79 min); however the difference was not statistically significant (p=0.052). These findings suggest that while ultrasound guided TAP blocks are as effective as laparoscopic TAP blocks; they may not be as efficient.
ARE ONLINE SYMPTOM CHECKERS ACCURATE FOR THE DIABETIC PATIENT POPULATION?: A REAL-LIFE EMERGENCY ROOM EXPERIENCE Hayden Hamby. Sponsor: Andrew C. Berry, DO, Dept. of Medicine, USAMC, Mobile, AL

Patients are now more health conscious and apt to using multimedia, such as online symptom checkers, to research their health. Limited studies to date have analyzed symptom checker diagnostic and triage accuracy for a subset of clinical illnesses. Critiques have called for consecutive, real-life, head to head comparison of symptom checker accuracy. We focused our analysis on diabetic patients—as diabetes is a rising worldwide health problem with high morbidity and mortality, harboring many diagnostic quandaries. Numerous studies have shown the challenging predictability in diabetic patients presenting symptoms, for example, those presenting with acute coronary syndrome do not present with classic chest pain symptoms, leading to initial diagnostic dilemmas. We aimed to assess the diagnostic and triage accuracy of medical online symptom checkers with regards to diabetic patients, using consecutive, real-life emergency room (ER) patient encounters. An ER chart analysis from Sept 2013-Sept 2015 within a selected block each month was performed, randomly selecting 1517 patients. Patients were classified as diabetics or non-diabetics. No distinction of diabetic type, treatment regimen, or severity was made. Exclusions included: transfers, patients found down/unconscious, major trauma, left early, or had a vague ER-listed diagnosis. 589 eligible patients remained: 85 diabetic and 503 non-diabetic. Numerous variables were collected: demographics, ER workup, admission status, diagnosis. Five top-volume online symptom checkers were utilized for diagnosis, three with triage capabilities. Symptoms were entered into online checkers by study personal. Physician-determined diagnosis (gold standard) was compared to symptom checker diagnostic output for analysis. 85 of 589 (14.4%) of patients analyzed were diabetic. Between diabetic and non-diabetic groupings, no significant differences were noted between gender or race, although diabetics were significantly of mean older age (p<0.001). For Symptomate and Symcat, respectively, diabetic patients had significantly worse diagnostic accuracy compared to non-diabetics [(Listed at all: 20.0% to 31.4%, 44.7% to 57.3%); (Top1: 14.1% to 24.1%, 20.0% to 26.0%); (Top3: 18.8% to 30.2%, 35.3% to 46.9%), (Top10: 20.0% to 31.2%, 44.7% to 56.9%). Isabel, MayoClinic, and WebMD showed non-significant trends of inferior diabetic diagnostic accuracy for the majority of the categories. No significances were noted between diabetics and non-diabetics for emergent triage accuracy for any of the checkers. Minimal to weak (k 0.21-0.59) agreement existed among the 5 symptom checkers for diagnostic accuracy (Cohen's agreement), with minimal to moderate (k 0.21-0.79) agreement for triage accuracy. ER utilization of labs, medications, prescriptions, imaging, consultants displayed no differences between the two groups. Admission to hospital was significantly different, as 36.5% of diabetics vs. 22.9% non-diabetics were admitted to the hospital (p=0.01), although there was no difference in length of stay. Of those admitted, a non-significant difference was noted in initial diagnosis and discharge diagnosis agreement, with diabetic diagnosis agreement 58.1% and non-diabetic agreement 72.7%. Overall, symptom checkers remain inferior diagnostic and triage modalities compared to physician-determined diagnosis and symptom triage. Diabetics appear to have worse symptom checker diagnostic accuracy, possibly stemming from their atypical symptom presentations.
ABSTRACT


Introduction: Sickle cell disease (SCD) is a genetic disorder caused by a single amino acid substitution of valine for glutamic acid in the sixth position of the beta chain on chromosome 11 of the hemoglobin tetramer. The pathophysiology of SCD involves microvascular occlusion related to polymerization of deoxyhemoglobin S promoted by regional hypoxemia and tissue hypoxia. Microvascular occlusion leads to tissue injury such as, acute chest syndrome, stroke, pulmonary hypertension and sickle nephropathy. Protein wasting in the urine is an early sign of glomerular damage to the kidney. Research Problem: macroalbuminuria and proteinuria are sensitive markers for glomerular damage and may be an early predictor for the development of chronic kidney disease in SCD. The reno-protective properties of ACE inhibitors and ARBs have been shown to be effective in a variety of clinical conditions and their short term (6 months) use in sickle hemoglobinopathies suggest the same. The USA Comprehensive Sickle Cell Center follows 204 patients of which 57 adults patients have demonstrated macroalbuminuria (> 300mg Pr/g Cr). Objective: To determine the effectiveness of ACE inhibitors and ARBs on decreasing macroalbuminuria in an adult cohort of individuals with a sickle hemoglobinopathy. Experimental protocol: This was a retrospective chart review of 57 adult (≥ 19) patients with SCD evaluated at the University of South Alabama Sickle Cell Center during the 5 year period between January 1, 2011 and December 31, 2016 with macroalbuminuria treated with an ACEi/ARB. All genotypes were included (HbSS, HBSB^thalassemia, HBSB^+-thalassemia, HbSC). Macroalbuminuria was determined based on urine albumin: creatinine ratio of >300mg Pr/g Cr. The urine protein:creatinine was determined at baseline and q6 months x 4. In addition, the hemoglobin, creatinine, GFR, and blood pressure were monitored across the same time intervals. Patients on hydroxyurea were not excluded from this study. Data was evaluated in SPSS and analyzed using nonparametric t tests as well as the Wilcoxon Signed Rank Test when within group differences occurred. Data was expressed as mean ± Standard Deviation. Data was considered significant when the p value was ≤ 0.05. Results: 1. In this cohort, macroalbuminuria prevalence was 28% 2. There was a significant reduction in proteinuria after 6 months of therapy (p= 0.009). There was no significant difference in protein:creatinine ratio at 12 and 18-24 months as compared with baseline. 3. When comparing GFR, creatinine, blood pressure, and hemoglobin, no significant difference was demonstrated from baseline to 18-24 months of the study. Conclusion: These results suggest a potential benefit of using an ACEi or ARB for sickle cell patients demonstrating macroalbuminuria. Further studies adequately designed and powered to address efficacy of ACEi/ ARBs are needed.
ABSTRACT

NOVEL SNORNAS CONTRIBUTORS TO MALIGNANCY Aliyah Kennedy
Sponsored by Glen Borchert, Ph.D., Department of Biology, College of Arts and Sciences, Mobile, AL.

Small non-coding RNAs have been at the forefront of genetic research following the discovery that these molecules participate in post-transcriptional gene regulation. Small nucleolar RNAs (snoRNAs) have long been thought to primarily function as chemical modifiers of other RNAs; however, snoRNAs have recently been found to exhibit differential expression between normal and cancer tissues and what’s more to function similarly to microRNAs. The Borchert Lab has recently published findings that snoRNAs produce smaller, miRNA-like fragments called sno-derived RNAs (sdRNAs), which are capable of regulating gene expression through complementary base pairing. In a recent study, we analyzed two breast cancer cell lines (MCF-7 and MDA-MB-231) for differential expression of snoRNAs. Excitingly, we found sdRNA-93 to be significantly more highly expressed in MDA-MB-231, and that high levels of sdRNA-93 could enhance cellular proliferation and invasion. These preliminary findings led us to profile sdRNA levels across several hundred publically available next generation sequence read archives (SRAs) corresponding to small RNAs from various cancer and normal tissues.

In this project, we have attempted to determine novel sdRNA contributions to malignancy, and produce a catalog of recurring sdRNAs in both malignant and normal tissues. Using a novel bioinformatics strategy we have now successfully characterized 167 new sdRNAs potentially relevant to an array of malignancies. In our analysis we found that sdRNAs showed a bias for malignancy based on cancer type. For example, we found 16 sdRNAs were uniquely and consistently overexpressed in lung malignancy compared to normal tissue, 6 sdRNAs were overexpressed in colon cancer, and 7 sdRNAs were overexpressed in melanoma. We also report >50 unique sdRNAs that exhibit a >10 fold increase in expression during malignancies of all types. These findings will allow us to determine the specific contributions of individual sdRNAs to specific pathologies and potentially lead to the development of new cancer diagnostic and therapeutic tools.
LOSS OF XRCC1 PROVIDES INCREASED CANCER CELL SENSITIVITY TO CHEMOTHERAPEUTICS & RADIATION.  G. Reid McClenny.  Sponsored by Jennifer Clark, Ph.D., Peter Sykora, Ph.D., Joel Andrews, Ph.D., and Robert W. Sobol, Ph.D, Department of Oncologic Sciences, Mitchell Cancer Institute, University of South Alabama College of Medicine, Mobile, AL.

One limitation of chemotherapeutics and radiation efficacy is the DNA repair capabilities of malignant cells. XRCC1 has been shown to be a critical scaffold protein involved in DNA repair, providing an essential role in the pathway of Base Excision Repair. The goal of this project is to examine the loss of XRCC1 function in U2OS (osteosarcoma) cells and to determine whether cancer cells lacking this key DNA damage repair protein become more sensitive to chemotherapeutic agents and radiation. Using the CRISPR/Cas9 approach, lentiviral plasmids containing the gene editing enzyme, Cas9, and specific guide RNAs were transduced into U2OS cells. Protein knockout was confirmed via immunoblot analysis. To measure DNA damage in the cells, we analyzed the cells via the CometChip assay, which measures genomic DNA damage in single cells. CometChip analysis revealed an increase in the baseline level of DNA damage as well as a defect in the capacity to repair damage induced by MNNG (alkylation agent) and hydrogen peroxide in cells lacking XRCC1. Further, we evaluated the recruitment of DNA repair proteins using confocal microscopy combined with laser micro-irradiation induced DNA damage. We found that in the absence of XRCC1, the partner protein DNA Polymerase beta was not able to be recruited to DNA damage sites induced by laser micro-irradiation. Our findings show that the loss of XRCC1 in U2OS cells results in increased levels of DNA damage and a defect in cellular DNA repair capacity.
ROLE OF ENDOTHELIAL TRPV4 CHANNELS IN CAROTID ARTERY FUNCTION AND LOW-FLOW REMODELING. Stuart McFarland. Sponsored by Mark S. Taylor, Ph.D. and David Weber, Ph.D., Department of Physiology and Cell Biology, University of South Alabama College of Medicine, Mobile, AL.

Obstructive cardiovascular disease (e.g. atherosclerosis) is associated with reduced blood flow, progressive endothelial dysfunction and vascular remodeling. Understanding how the endothelium shifts from its normal function to a state of dysfunction is important in preventing the development of the disease. Current evidence suggests a pivotal mechanism may involve endothelial TRPV4 cation channels that mediate vascular shear stress and vasodilator responses by controlling endothelial Ca\(^{2+}\) signaling. In the current study, we assessed the specific impact of endothelial TRPV4 channels on carotid artery function in both wild type control and endothelial-specific TRPV4 knockout transgenic mice (KO). Following tissue harvest, carotid arteries were cut into rings for isometric force measurements via myography. Contractile responses were induced with 60 mM KCl which depolarizes the smooth muscle cells, or with phenylephrine (10\(^{-6}\) -10\(^{-4}\) M), an \(\alpha_1\) adrenergic receptor agonist. To determine dilator responses, arteries were half-maximally contracted with phenylephrine and then relaxed with acetylcholine (10\(^{-9}\) -10\(^{-4}\) M), a muscarinic agonist which causes the endothelium to release nitric oxide. After completion of acetylcholine-induced relaxation, treatment with sodium nitroprusside (10 mM), a nitric oxide donor that causes endothelium independent relaxation, removed any remaining contractile tone. Depolarization-induced contractions (60 mM KCl) were larger in KO than WT (1.14 ± 0.14 mN vs. 0.52 ± 0.08 mN, P<0.0005, N=6) and a similar trend was noted for phenylephrine-induced contractions (0.68 ± 0.12 mN vs. 0.43 ± 0.08 mN, P<0.1, N=6). The EC\(_{50}\) calculated phenylephrine showed no significant difference (1.7x10\(^{-7}\) vs 1.5x10\(^{-7}\) M, n=6) in sensitivity. There was no significant difference in either the maximal relaxation (67±7% vs 67±9%, n=6) or in the EC\(_{50}\) of the acetylcholine induced relaxation (5.9x10\(^{-7}\) vs 4.6x10\(^{-7}\), n=6) between KO and WT carotids. Overall, our findings suggest that loss of endothelial TRPV4 channels inherently increases vascular contractility independent of a receptor pathway while the endothelium-dependent relaxation is largely unaffected.
ACCESS TO EMPLOYER PROVIDED HEALTH INSURANCE IN LOW INCOME COMMUNITIES. Destini Smith. Sponsored by Kenneth Hudson, Ph.D., Department of Sociology, Anthropology, and Social Work, University of South Alabama, and Errol Crook, M.D., Abraham A. Mitchell Professor and Chair, Department of Medicine, University of South Alabama College of Medicine, Mobile, AL.

Data from the Annual Social and Economic Survey indicates that the majority of adult Americans with health insurance obtain their coverage through their employer or union. Prior research, however, suggests that individuals in low income communities are less likely to have jobs that provide them with health insurance than people who live in more affluent neighborhoods. In this study, we use data from the Labor Market Health Care Survey, 2006-2017 (N=231), to examine the likelihood that individuals in high poverty census tracts in Mobile County, Alabama, will obtain a job that provides them with health insurance. We use survival analysis to estimate the proportion of this population that will transition during their life-course into a job with employer provided health insurance. Although we expect that most Americans who transition to a job with health insurance will continue to have a job or jobs that provide this benefit until they retire, we examine whether or not this is true for the individuals in our study.

The data used in this study was collected using a two-stage probability sample. The low income population in this study is defined by census tracts where 50% or more of the residents have family incomes below the federal poverty threshold. In these communities, there is a strong correlation between percent poor and percent black ($rho = .774, p < .000$). Consequently, about 98% of our randomly selected participants are African American.

RESULTS: First, our examination of the survival functions shows that approximately 37% of adults in this population never obtain a job with employer provided health insurance. Although there is not a significant sex difference in the survival functions, those individuals who do not finish high school are much less likely than others to ever obtain a job that provides health care coverage (the modal category of educational attainment is high school diploma). We also find that 63% of adults who do transition into a job with health insurance are not able to sustain employment that provides health care coverage until they reach the minimum retirement age at 62.
ABSTRACT

HYPERSPECTRAL IMAGING OF INSULIN-SECRETING INS-1E CELLS. Supraja “Sippy” Sridhar. Sponsored by Thomas Rich, Ph.D., Department of Pharmacology, and Silas Leavesley, Ph.D., Department of Chemical Engineering, Center for Lung Biology, University of South Alabama College of Medicine, Mobile, AL.

Cyclic adenosine monophosphate (cAMP) is a second messenger that regulates many physiological processes. The ubiquitous nature of cAMP has spawned studies to understand how the information required to orchestrate these processes is encoded within cAMP signals. Recent studies suggest that measuring total cellular cAMP concentration in cell populations is not sufficient to characterize intracellular signaling pathways including the cAMP signaling pathway.

To overcome these limitations, we monitored the time course of cAMP signals using FRET-based cAMP probes transfected in insulin-secreting INS-1 cells. INS-1 cells are the most frequently used cell line for insulin secretion studies and are derived from rat insulinoma, a benign tumor of pancreatic beta cells. FRET was measured using hyperspectral imaging approaches developed in the Leavesley and Rich groups. This method provides information on the subcellular location of cAMP signals within INS-1 cells. In these experiments cAMP synthesis was triggered by addition of either GLP-1 or glucose challenge.

INS-1 cells were transfected with the H188 FRET-based cAMP probe. Two days following transfection the extracellular buffer was replaced with a low glucose (2 mM) buffer for 1-2 hours and the loaded with the nuclear label DRAQ5. Cells were imaged using the spectral mode of the Nikon A1R confocal microscope. The results demonstrate that both GLP-1 and glucose challenge trigger cAMP synthesis in the perinuclear region of INS-1 cells. We then tested whether inhibitors of soluble adenylyl cyclase (LRE), clatherin-mediated endocytosis (Dyngo-4a), or phosphodiesterase activity (IBMX, rolipram, or trequisin) alter the subcellular distribution of GLP-1 or glucose challenge mediated cAMP responses. We observed that pretreatment with Dyngo-4a or LRE had little or no effect on GLP-1 or glucose challenge-mediated cAMP responses. Treatment of INS-1 cells with IBMX revealed cAMP production occurs under baseline conditions, consistent with a futile cycle of cAMP synthesis and hydrolysis. In contrast, little or no cAMP accumulation was observed following treatment with PDE4 inhibitor rolipram. Taken together, these results indicate that the kinetics and spatial distributions of cAMP signals in INS-1 cells are complex. In the future we plan to examine these signals on imaging systems with higher spatial and temporal resolution.
ABSTRACT

DEPLETION OF MITOCHONDRIAL DNA: IMPACT ON BIOENERGETICS AND Ca^{2+} SIGNALING IN LUNG MICROVASCULAR ENDOTHELIUM. Galen Garriga. Sponsored by Mikhail Alexeyev, Ph.D. and Mary Townsley, Ph.D., Department of Physiology and Cell Biology, University of South Alabama College of Medicine, Mobile, Alabama.

Endothelial barrier function is tightly regulated by cytosolic Ca^{2+} concentration, which is determined by the balance between Ca^{2+} flux at the plasma membrane and intracellular Ca^{2+} sequestration. Ca^{2+} dynamics are currently interpreted based on global Ca^{2+} function in a population of endothelial cells, but accumulating evidence suggests that functional outcomes may be more closely associated with focal Ca^{2+} dynamics and microdomains partially formed by mitochondrial Ca^{2+} uptake. If true, we postulate that mitochondrial dysfunction should lead to disruption of these domains and Ca^{2+} signaling. We have shown that in pulmonary microvascular endothelial cells (PMVECs), mitochondrial complex I inhibition impairs cytosolic Ca^{2+} handling after activation of plasma membrane TRPV4 channels. Further, we have developed a ρ^{0} PMVEC line depleted of mitochondrial DNA (mtDNA) by treating wild type PMVECs with ethidium bromide (EtBr) and dideoxycytidine (ddC). The goal of this study was further characterize ρ^{0} PMVEC cells and cytoplasmic hybrids (cybrids), the latter derived by reintroducing mtDNA into ρ^{0} PMVEC cells. To accomplish this goal, ρ^{0} PMVEC were fused with chemically enucleated PMVEC/2641 (wild type donor PMVEC marked by insertion of rtTA gene in nuclear DNA) using polyethylene glycol thus generating cybrids. Using PCR for rtTA, we confirmed the lack of this nuclear genetic marker in the cybrids thus confirming their nuclear derivation from recipient ρ^{0} cells. Using electron microscopy, we found that ρ^{0} cells have abnormal mitochondrial morphology compared to the wild type PMVEC, and are currently waiting for images of cybrid cells. We will be measuring mitochondrial mass and membrane potential by flow cytometry using the fluorescent markers 10-N-nonyl acridine orange and tetramethylrhodamine, respectively. We are still in the process of completing the Ca^{2+} dynamic analysis for wild type PMVECs, ρ^{0} PMVECs, and cybrids loaded with the Ca^{2+} fluorophore Fluo-8AM and treated with the TRPV4 4αPDD. To that end, we will use the LC-Pro region of interest tracking script for ImageJ and ROI analysis script in R (statistical computing language). While cellular morphology in PMVEC cybrids, at least at the level of light microscopy, appears to have normalized compared to that in ρ^{0} PMVECs, conclusions regarding mitochondrial function and Ca^{2+} dynamics must await completion of remaining analyses.
**ABSTRACT**

γδ T cells as a potential vaccine target for intracorneal HSV-1. Jack Friend. Sponsored by Robert Barrington, Ph.D., and Robert Lausch, Ph.D., Department of Microbiology and Immunology, University of South Alabama College of Medicine, Mobile, AL.

Intracorneal (i.c) herpes simplex virus (HSV-1) is the leading cause of infectious blindness in developed countries around the world, including the United States. Traditionally, αβ T cells have been thought of as the main T lymphocyte present and acting after a viral infection. However, recent findings have shown that γδ T cells may play a bigger role than originally thought in the defense against i.c. HSV-1 infection, in particular within the first 48 hours post-infection. Our data show that mice without γδ T cells succumb to encephalitis after being infected with HSV-1. We hypothesize that γδ T cells limit HSV-1 in the early stages of i.c infection through production of IL-17A. Moreover, we hypothesize that local production of chemokine CCL20 and cytokine IL-1α is required to recruit and activate γδ T cells to produce IL-17A.

Using a well-established mouse model of i.c. HSV-1 infection, we have examined production of CCL20, IL-1α, and IL-17A using qPCR and/or ELISA. While CCL20 mRNA was detectable in uninfected corneas, the levels were increased in corneas 24 hours after initial HSV-1 infection. To test the role of CCL20 in immunoprotection early after HSV-1 infection, we compared the responses in wild type mice to those of mice lacking CCR6, the ligand for CCL20. Using a scoring criteria from 1-5, where 5 represents the greatest corneal pathology, we found that CCR6-deficient mice had increased pathology compared to wild type mice. Examination of immune cell corneal infiltrates using flow cytometry revealed that CCR6-deficient mice had reduced γδ T cells and neutrophils compared to wild type mice. Because IL-17A is key to neutrophil recruitment in mouse cornea post-infection, the observed reduction in neutrophils in CC6-deficient mice is consistent with reduced IL-17A production. To begin to determine whether IL-1α is important for activating γδ T cells entering the cornea, we compared the levels of IL-1α in uninfected and infected wild type and CCR6-deficient mice. We found that IL-1α levels increase in wild type and CCR6-deficient mice at the time points 15 and 24 hours post-infection relative to levels in uninfected cornea.

Future studies will test whether resident or recruited γδ T cells are necessary for protection against i.c. HSV-1 infection. We propose that understanding these early pathways of protection by γδ T cells will provide a potential vaccine target to strengthen early responses to i.c. HSV-1, thereby limiting corneal damage.
ABSTRACT

VALIDATION OF THE DETECTION OF BRAF, KRAS, NRAS, & EGFR MUTATIONS IN MELANOMA, COLORECTAL, AND LUNG CANCERS USING A REAL-TIME PCR ASSAY. Travis B. Goodloe, III. Sponsored by Brett Baskovich, M.D., Department of Pathology, University of South Alabama Medical Center, Mobile, AL.

Melanoma, colorectal cancers, and lung cancers often originate from mutations in oncogenes that ordinarily promote proper cell function and proliferation. Clinical detection methods of such mutations have largely relied on sequencing techniques, chiefly pyrosequencing, that allow for detection with 5-10% sensitivity. Due to factors such as tumor heterogeneity and the presence of minimal tumor among normal tissue, mutations may go undetected and thus untreated. Real-Time PCR utilizes mutation-specific probes that allow for monitoring of DNA amplification with an expected lower limit of detection. Previously pyrosequenced cases were identified and reviewed from the USA Department of Pathology. Twenty positive and twenty negative cases of BRAF, KRAS, NRAS, and EGFR were selected including a number of cases from outside institutions. DNA was extracted from formalin-fixed paraffin-embedded (FFPE) tissue for all suitable cases, and Real-Time PCR analysis was performed. Importantly, a dilution assay demonstrated a more sensitive, lower limit of detection on a BRAF V600E positive case at a value of 0.1% compared to previous pyrosequencing results. Ultimately, validation on all selected cases has confirmed that the Real-Time PCR assay for BRAF and KRAS mutations in melanoma and colorectal cancers reaches a lower limit of detection than previous methods with similar accuracy. The research is now focusing on NRAS and EGFR mutation detection. Two presumably NRAS negative cases have demonstrated a possible G12D mutation that was not indicated by pyrosequencing. Consultation from an additional party using a third method of detection is now needed to determine if these two cases are false positives or novel G12D mutation detections. If truly positive, strong evidence would be in place supporting the efficacy of Real-Time PCR over pyrosequencing for NRAS mutation detection. Similarly, Real-Time PCR EGFR mutation detection also has shown significant promise, but the probe for detection of a nine base pair insertion by the Real-Time assay has been overactive, leading to potential instances of false positives. Adjustments to the baseline in the ABI 7500 software have largely alleviated many of these cases, but some cases are currently outstanding. At this time, additional runs on the Real-Time instrument as well as outside consultation are needed for full resolution. Ultimately, once validation of this Real-Time PCR cancer mutation detection assay is completed, it will be utilized clinically on active patient cases in the Department of Pathology at the USA Medical Center as a replacement to less sensitive pyrosequencing.
ABSTRACT

NOT ALL COLLOIDS ARE CREATED EQUAL: PLASMA RESCUE DECREASES FLUID CREEP DURING BURN RESUSCITATION COMPARED TO ALBUMIN. Sara Anne Stringfellow. Sponsored by Steven Kahn, M.D., and Sidney Brevard, M.D., Department of Acute Care Surgery and Burns, University of South Alabama Medical Center, Mobile, AL.

Significant fluid resuscitation is required in the treatment of severe burns due to loss of fluid barrier integrity and capillary leak. Striking a balance is critical, as “fluid creep” and over-resuscitation are as problematic as under-resuscitation. Body composition poses a challenge in these patients, as obese patients have a higher ratio of adipose tissue with decreased vascularization. However, this excess weight is still included in traditional fluid resuscitation formulae. Recent literature has found that fresh frozen plasma (FFP) restores glycocalyx integrity and reverses capillary leak in patients with shock. Our study compares historical controls resuscitated with Parkland-based resuscitation and albumin with an adjusted ideal body weight (AIBW) index formula with FFP rescue. This is a retrospective review of adult burn admissions with >20%TBSA burns from 1/2010-5/2017. Patients were only included if they were between the ages of 18 and 79 and they survived over 48 hours after admission. Historical controls were resuscitated with the Parkland formula and albumin rescue, periodically titrated to urine output. AIBW patients were resuscitated with the ABA consensus formula with an AIBW Index with rescue FFP if oliguric for more than 2 hours. Demographics and outcomes were compared using non-parametric statistics. There were 145 total patients in the study. The AIBW group (N=31) required significantly less fluid in the first 24 hours than the control group (N=114) (2.9 cc/kg/%TBSA vs 4.3 cc/kg/%TBSA) (p=<0.0001). The AIBW group also produced significantly less urine (1 vs 1.4 cc/kg/hr) (p<0.001) while showing a trend for fewer ventilator days (3 vs 6 days) (p=0.25) and lower mortality (3.2% vs 20%) (p=0.10). When comparing only those patients that received FFP rescue on the AIBW formula (N=15) compared with all controls that received albumin (N=75), the AIBW group required less fluid overall (3.79 vs 4.59 cc/kg/%TBSA) (p<0.05) and produced less urine (1.16 vs 1.56 cc/kg/hr) (p=0.0052). There was a trend toward AIBW patients being less likely to develop acute kidney injury requiring dialysis (6.7% vs 29.3%) (p=0.10). The AIBW formula with FFP rescue appears to be a safe and effective alternative to the Parkland formula in burn resuscitation. AIBW patients received less fluid than Parkland formula patients without an increase in acute kidney injury. This preliminary data suggests a trend toward fewer ventilator days and lower mortality, but a larger sample size is required to fully elucidate the differences between these two treatment strategies.
COMPARISON OF TIDAL VOLUMES OF MECHANICALLY VENTILATED TRAUMA PATIENTS BEFORE AND AFTER THE IMPLEMENTATION OF THE MECHANICAL VENTILATION PROTOCOL. Hannah Ficarino. Sponsored by Sidney Brevard, MD, Department of Acute Care Surgery and Burns, University of South Alabama Medical Center, Mobile, AL.

Acute respiratory distress syndrome (ARDS) is a significant contributor to morbidity and mortality after traumatic injury. High tidal volume ventilation can cause iatrogenic volutrauma and barotrauma in the lungs, but this is combatted by use of low tidal volume ventilation after development of ARDS or as a possible prevention strategy for those at risk of developing ARDS. The aim of our study was to determine if implementation of a low tidal volume ventilation protocol (<6mL/kg of predicted body weight [PBW]) resulted in lower tidal volumes on the surgical-trauma intensive care unit (STICU). A retrospective review of a pre- and post-protocol group was performed on patients requiring invasive mechanical ventilation after admission to the trauma service. A chart review of patients discharged from January 2015 through March 2017 was performed to compare initial tidal volume, lowest and highest tidal volume, and initial ventilation mode used in a pre-protocol cohort (July 2015-April 2016) and post-protocol cohort (July 2016- March 2017). Time at goal tidal volume (<6 mL/kg of PBW) and total hours ventilated were compared between the two groups, along with injuries and complications. Implementation of the protocol was associated with a significant decrease in initial tidal volume (503.6 ± 29.0 vs. 441.5 ± 67.5, p<0.0001), lowest tidal volume used (477 ±45.5 vs. 427.5 ±44.1, p<0.0001), and highest tidal volume used (517.5 ± 37.9 vs. 459.4 ± 54.0, p<0.0001). A shift in the initial ventilation mode used from pre-protocol (SIMV 90.7%) to post-protocol (VC 89.0%) was also found. Though most complications did not show a significant change, there was a decrease in aspiration pneumonia from pre- to post-protocol (4.5% vs. 1%, p=0.03). There was not a significant decrease in hospital days, ventilator days, or intensive care unit days. These findings suggest that implementation of a mechanical ventilation protocol on the STICU was associated with a difference in pre- and post-management strategies.
INVESTIGATION OF THE SIGNIFICANCE OF SON ISOFORM mRNA EXPRESSION IN PATIENTS WITH BLOOD CYTOPENIAS WITH/WITHOUT EVIDENCE OF MYELOID NEOPLASIA. Aaron Dinerman. Sponsored by Thomas Butler, M.D., Department of Medical Oncology, Mitchell Cancer Institute and Erin Ahn, Ph.D., Department of Oncologic Sciences, Mitchell Cancer Institute and Department of Biochemistry and Molecular Biology, University of South Alabama College of Medicine, Mobile, AL.

Myelodysplastic Syndrome (MDS) is a clonal hematopoietic disorder related to ineffective hematopoiesis and hematopoietic stem cell dysplasia. Cytogenetic information regarding MDS to help guide diagnosis and prognostics is limited; therefore, further research into new tools and biological targets for this disease entity are of significant interest to the medical community. While major RNA splicing components have been found to be key players in the pathogenesis of MDS, there is not a large reservoir of data for the use of such pathophysiology in the clinical care of MDS. SON Protein has been described as an RNA splicing co-factor and transcriptional repressor that regulates expression of multiple genes involved in cell cycle progression, stem cell pluripotency maintenance, and leukemogenesis. We have recently discovered that SON short isoforms (SON E and SON B) generated by alternative RNA splicing are aberrantly upregulated in MDS and AML. Considering the splicing pathophysiology that may be involved in MDS, we designed a study that would investigate the variable expression of SON isoforms in a variety of clinical circumstances related to myeloid disorders. It was originally postulated that different SON short isoforms (both SON E and SON B) might exhibit a progression in myeloid dysplasia/neoplasia because MDS occasionally progresses into AML. However, it was decided that myeloproliferative neoplasms, such as Polycythemia Vera (PV), might also demonstrate a SON-manifested pathophysiology. We collected Peripheral Blood Mononuclear Cells (PBMCs) and Bone Marrow specimens (BM) from consented patients at Mitchell Cancer Institute that were identified as ideal candidates for inclusion based upon some degree of myeloid pathology. Investigation with RNA extraction and RT-qPCR demonstrated a noticeable difference in SON isoform mRNA expression in all patient samples obtained with comparison to normal/healthy donor PBMCs. Patient samples included diagnoses of MDS, anemia, neutropenia, PV, and AML (all myeloid hematopoietic pathologies). SON E levels were significantly elevated compared to normal patient samples. Additionally, considerable differences were noted in SON B isoform expression. These findings demonstrate an interesting and potentially noteworthy correlation between SON mRNA alternative splicing and the pathophysiology of myeloid hematopoietic disorders. Although further research is necessary to confirm a relationship and illuminate its specific significance, this discovery is an early promising link between the biology of splicing dynamics and the clinical manifestations of myeloid neoplasia.
TRUAMA PATIENTS HAVE IMPROVED ACCESS TO POST-DISCHARGE RESOURCES THROUGH THE AFFORDABLE CARE ACT. Patricia Connor. Sponsored by Sidney Brevard, MD; Ashley Williams, MD; S. Noelle Davis, CRNP, Department of Surgery, University of South Alabama Medical Center, Mobile, AL.

The state of Alabama is one of 19 states opting out of Medicaid expansion under the Affordable Care Act (ACA). Previous studies in Medicaid-expanded states have demonstrated up to a 20% decrease in their uninsured trauma population. The purpose of our study was to evaluate our trauma population payer mix before and after the implementation of the ACA and its effect on disposition. This was a retrospective review of adult trauma patients treated at the University of South Alabama Medical Center (USAMC) level I trauma center serving a large rural catchment area between 2011-2012 (pre-ACA) and 2014-2015 (post-ACA). Payer status, insurance type, demographics, injury severity score, type of trauma, and patient disposition were recorded. We identified 3716 patients in the pre-ACA period and 3657 patients in the post-ACA period. Patients in the post-ACA period were more likely to be insured (55.14% vs 59.37%, p<0.001) and utilize post discharge services (9.28% vs 13.48%, p<0.001) when compared to the pre-ACA period. Medicaid patients are more likely than uninsured patients to receive home health services (OR 6.24, CI 2.74-14.18) or intermediate nursing care (OR 16.88, CI 7.05-40.43). After the implementation of the ACA, the USAMC trauma center experienced a statistically significant decrease in the overall number of uninsured trauma patients with increased utilization of extended services after discharge. However, in our state without Medicaid expansion, this still leaves 40% of our trauma patient population without health insurance coverage.
ABSTRACT

TREATMENT OF HEPATITIS C INFECTION IN HIV INFECTED PATIENTS IN INNER CITY CLINICS. Verlisa Kennedy. Sponsored by Eduardo Calderon, M.D., Division of Infectious Diseases, University of South Alabama, Mobile, AL.

In the United States, there are between 3 and 5 million people infected with chronic Hepatitis C Virus (HCV) and about 1.3 million people infected with Human Immunodeficiency Virus (HIV). It is estimated that 25% of those infected with HIV are co-infected with HCV. Co-infection of both viruses has high frequencies of detrimental effects on a variety of human cells, such as hepatocytes, lymphocytes and nephrons. Treatment of the HCV infections simultaneously with the management of antiretroviral therapy for HIV control should prevent advancement of liver fibrosis and preserve liver function and the function of other organs.

Using the electronic medical records (EMRs) from eligible patients from Franklin Primary Health Clinic and the Mobile Department of Public Health, in Mobile, Alabama, data was collected and analyzed. The data included but was not limited to the following parameters collected before treatment, during treatment, end of treatment and the sustained virologic response (SVR) at 12 weeks: Age, race, HIV and HCV therapies, CD4 lymphocyte count, creatinine, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) values. Forty-seven HIV/HCV co-infected inner city individuals initiated therapy: 1 with Sofosbuvir (SOF) and Ribavirin (RBV), 1 with SOF and Peg-interferon (PEG), 15 with SOF+RBV+PEG, 26 with SOF and ledipasvir (Harvoni), 1 with SOF and velpatasvir (Epclusa), and 3 with elbasvir and grazoprevir (Zepatier). The total number of evaluated patients in this preliminary report was 47 with 38 patients having a SVR12. Overall SVR12 was 94.8% (36/38).

In regards to race, 70.2% (33/47) were African American 29.7% were Caucasian (14/47). The SVR12 collected showed a mild difference between African Americans 92% (24/26) and Caucasians 100% (12/12). The number of patients with available CD4 lymphocyte count prior to treatment was 46. There were 6.5% (3/46) of patients’ CD4 < 200 cells, patients with CD4 of 201-499 cells 34.7% (16/46) and CD4 >500 cells at 58.69% (27/46). At the time that SVR12 was checked, the patients who had an available lymphocyte count were 35. Out of those 35 patients, 0% (0/35) had a CD4 < 200 cells, 25.7% (9/35) had a CD4 of 499-201 cells and 74.2% (26/35) had a CD4 > 500 cells. At time of SVR12, there was an increase in CD4 lymphocyte count in all three intervals. The number of patients with available serum creatinine, ALT and AST at SVR12 was 37. Examination of creatinine values at SVR12 showed a lower creatinine in 43% (16/37) of patients. ALT and AST also showed lower serum levels of 94.5% (35/37) and 89.31% (33/37), respectively. There was a marked decrease in liver associated enzymes suggestive of a decrease in liver dysfunction.

In our sample, the treatment of HCV infection in HIV co-infected patients shows improvement in renal and liver functions as well as a 94.8% success rate of individuals treated during this study becoming cured of HCV infection.
ABSTRACT

HIGHWAY-RAIL CROSSING INJURIES IN ALABAMA: GATES ARE NOT ENOUGH. Natalie Hargrave, BS sponsored by Jon Simmons MD; Steve Kahn, MD; Sid Brevard, MD MPH; Linda Ding, MD., Department of Surgery, University of South Alabama, Mobile, AL.

In 2014, Alabama ranked 4th in total Public Highway-Rail crossing collisions and 3rd in number of fatalities. There is a paucity of injury-prevention research on the determinants of these preventable deaths. We aimed to characterize the rail-crossing related injuries treated at our trauma center as well as evaluating state-wide data to identify targets for injury prevention. We hypothesized that the majority of accidents occur at crossings with insufficient safety devices. This was a retrospective review of patients treated at the University of South Alabama Medical Center from January 2006 to March 2017. We queried our trauma registry for all railway-related injuries and collected demographics, patient outcomes, and disposition. We also obtained detailed accident reports from the Federal Railroad Administration and examined the statewide injury pattern compared to our patient population. From 2006 to 2017, 48 patients were treated at our level 1 trauma center after a railway injury. 27 were motor vehicle collisions and 14 were pedestrian. Of the MVCs, 66% were men and the mean age was 36 years (11-69). The mean ISS for the MVC group was 18 (4-50) with 11% mortality rate. Mean length of stay was 9 days (1-50). 22% of patients required additional services after discharge. 41.6% of these collisions occurred at crossings with active safety devices which include bells, flashing lights and gates. This is consistent with statewide data demonstrating 49.3% of collisions occurring at crossings with active safety devices with nearly half resulting in injury or death. Motor vehicle collisions at railway crossings lead to significant morbidity and mortality and is nearly always preventable. For these injured patients, active safety measures at crossings are insufficient for injury prevention. Public awareness and education should be evaluated as an adjunct prevention measure.
Does Completion of a Fourth-Year Internal Medicine Sub-I Predict Success as a General Surgery Intern? Frank Foley. Sponsored by Linda Ding, M.D., Jon D. Simmons, M.D., F.A.C.S. Division of Acute Care Surgery and Burns University of South Alabama College of Medicine, Mobile, AL.

General Surgery Intern Year is a very demanding introduction into the general surgery 5-year residency program. Interns are expected to admit, manage patients, and take part in operations. This stress and responsibility can be easier for those who have experienced intensive 4th year electives in medicine. The retrospective study conducted analyzed the evaluation results of all South Alabama interns from 2005 to 2017 by scoring them in the six core competencies these metrics were totaled and their performance was evaluated against the number and type of electives taken during each respective interns fourth year. The data revealed
Establishment of a Lymphedema Registry for the University of South Alabama.
Kristin Sheehan. Sponsored by Donna Lynn Dyess, M.D., J. Spencer Liles, M.D.,
Department of Surgery, University of South Alabama, Mobile, AL.

Lymphedema is the accumulation of lymphatic fluid in the interstitial space, clinically
manifested as swelling. Breast cancer is the most common cause of lymphedema, resulting
from the disease itself and/or the treatments necessary to control the disease. Commonly,
patients will report a feeling of heaviness in the limb, tighter fitting rings, bracelets, or
clothing, or numbness/tingling of the limb. The progression of lymphedema is usually slow.
Patients may not notice any difference until later stages. The course of lymphedema is
progressive, with the beginning stages being reversible and the later stages resulting in
underlying structural changes. Once a patient develops mild lymphedema, they are more
likely to subsequently develop severe lymphedema than patients with no evidence of
lymphedema. Changes to skin are also seen, with affected areas being erythematous and
fibrotic. This may lead to infection, cellulitis, and necrotizing fasciitis. The development of
lymphedema significantly decreases the quality of life of the patient and may increase
psychological distress.

There are multiple ways to test for the presence of lymphedema, including water
displacement, infrared scanning, bioelectrical impedance measures, and tape
measurement, all of which have been found to be valid techniques for such assessment.
We opted for tape measurement due to reproducibility, ease of use, and minimal costs.
The goal of this research is to develop a database of baseline measurements that would
allow us to follow patients throughout their treatment processes and monitor for changes
that would suggest the development of lymphedema. This would allow for early detection
to minimize the effects of this complication and improve patient’s quality of life. The
database would also allow us to analyze when lymphedema is most likely to occur as well
as be used as a springboard for further research.

In our study measurements were taken at four locations on each arm: around the hand
from 2<sup>nd</sup> to 5<sup>th</sup> metacarpal-phalangeal joints, around the wrist including the distal ends of
the ulna and radius, 10 cm below the cubital fossa, and 10 cm above the cubital fossa.
Subsequent measurements were taken at the same locations at each step in the
management process. We defined clinical significance to be a 2 cm difference between a
patient’s left and right arm or a 2 cm difference in current measurements from the patient’s
baseline.

Timing of measurements taken were categorized based on the stage of treatment.
Categorizes included baseline, neoadjuvant chemotherapy, adjuvant chemotherapy,
radiation, surveillance less than one year, and surveillance greater than one year.

Over the course of the study we obtained 100 measurements and found 7 cases of
lymphedema. Two patients are in the course of adjuvant chemotherapy and five patients
fall into the surveillance greater than one year category.

Our preliminary results indicate that lymphedema is not as prevalent as stated in the
literature, although there are many inconsistencies. The National Cancer Institute
estimates the incidence as 8-56%. Our results indicate a rate of only 7%.
NUCLEAR LOCALIZATION OF POL λ: A ROLE FOR COTRANSPORT. William Wiggins
Sponsored by Natalie R Gassman, Ph.D. Department of Oncologic Sciences, Mitchell
Cancer Institute, University of South Alabama, Mobile, AL

DNA repair proteins play a critical role in ensuring the stability of our genome. These proteins use a variety of pathways to repair the damage we accumulate from environmental insults such as pollution, UV radiation or compounds in products such as tobacco. A necessary step in this process is the localization of repair proteins to areas of DNA damage. Nuclear localization signals (NLSs) support the active accumulation of proteins into the nucleus of cells, and they are contained in a large number of DNA repair proteins. However, the precise localization signals and other determinants that promote nuclear accumulation are still unknown for a large number of DNA repair proteins, including several member of the X family of DNA polymerases. In order to better understand the nuclear localization and DNA repair roles of one X family member DNA polymerase λ (Pol λ), we have characterized two regions of its unique non-catalytic N-terminal domain. This unique region is predicted to contain a nuclear localization signal and BRCT domain, which may mediate important DNA repair and localization protein interactions. A rational mutation strategy was employed to identify key residues in the NLS and BRCT domain that control nuclear accumulation. Selected Pol λ variants were transfected into cells, mouse embryonic fibroblasts (MEF) or human embryonic kidney (HEK293T) cells. Immunoblot and immunoprecipitation were used to verify protein expression and check for changes in protein interactions, respectively. Cells were also imaged with fluorescent microscopy to visualize alterations in localization patterns. Using these approaches, we showed the extent the NLS signal directs the localization of Pol λ and determined that the BRCT domain adjacent to the NLS can also direct some nuclear accumulation of the protein. Our data suggests that the role the BRCT region plays in accumulation is cotransport mediated by its Ku70 interaction. This is the first demonstration of a cotransport mechanism for Pol λ and suggests that nuclear localization of this protein is critical for genomic maintenance.
ABSTRACT

THE SIGNIFICANT IMPACT OF A DEDICATED TBL CURRICULUM ON A SURGERY CLERKSHIP. Patrick Steadman. Sponsored by Lee Grimm, Jr., M.D., FACS, FASCRS, Department of Surgery, University of South Alabama College of Medicine, Mobile, AL.

Team-Based Learning is a promising educational model that has gained popularity in recent years as a tool to enrich student experience and improve outcomes in undergraduate medical education. The Department of Surgery designed a new curriculum for the third-year surgery clerkship at The College of Medicine that featured 9 new Team-Based Learning modules, and student performance has subsequently improved. Through this study, we aimed to test the hypothesis that the new surgery clerkship curriculum emphasizing TBL was associated with the improvement in student examination scores and outcomes for the surgery clerkship. As our primary outcome, we collected results of the end-of-clerkship exam administered by the National Board of Medical Examiners and analyzed those results for classes both pre- and post-curriculum change. We also looked at results for the same classes on United States Medical Licensing Exam Step 2 CK, an exam taken after all third-year clerkships. Finally, we analyzed results on the Medical College Admissions Test (MCAT) and the USMLE Step 1 to normalize for potential baseline differences between the pre-intervention and intervention groups. We analyzed the Step 1, Step 2 CK, and Surgery NBME scores using a Z-test by the Central Limit Theorem, and we analyzed the MCAT scores using ANOVA. We found there to be a 55% improvement in the surgery clerkship national percentile ranks for students after the curriculum change, and we showed that the improvement was statistically significant (p<0.0001). We also discovered significant improvements on the Step 1 (p=0.009) and Step 2 CK Exams (p<0.0001) by the intervention population over the pre-intervention group. TBL appears to have had the most profound effect on the third-year portion of the curriculum, with the effect size of the results being larger for the Surgery NBME Exam (effect size=8.434) and the Step 2 CK Exam (effect size=4.273) than the Step 1 Exam (effect size=2.233). We determined that the MCAT scores for the students learning under the new TBL curriculum were not different than those learning under the older clerkship curriculum format (p=0.381). Thus, the MCAT results indeed acted as a negative control and confirmed that there was no baseline difference between the two groups of students when they began medical school. The content area item analysis of the surgery clerkship exam established that the exam itself was virtually identical for each of the study populations and demonstrated that Team-Based Learning aided students comprehensively across the different organ systems and physician-tasks being assessed. We concluded that the new curriculum emphasizing Team-Based Learning was associated with the student improvement in the surgery clerkship, and that it immensely contributed to student success across the first three years of medical school.
HEALTH INSURANCE DISPARITIES IN COLON, RECTAL, AND ANAL CANCER PATIENTS. Anna Stevens. Sponsored by Leander Grimm, MD, Department of Surgery, University of South Alabama, Mobile, AL.

It is estimated that there will be 135,430 new cases of colorectal cancer and 8,200 new cases of anal cancer diagnosed in the United States in 2017, with an estimated 50,260 and 1,100 deaths attributed to the diseases, respectively. In Alabama, the incidence rate of colorectal cancer was 44.2 per 100,000 people in 2014. While colorectal cancer remains the third most common cancer among men and women, both the incidence and death rates associated with colorectal cancer have decreased over the last 10 years. Improved screening rates has been implicated as a factor in reducing rates of incidence and mortality. Still, demographic and health insurance disparities in screening, incidence, and mortality rates remain. The aim of this study was to identify demographic and health insurance disparities in newly diagnosed colon, rectal, and anal cancer patients. An IRB approved retrospective chart analysis was performed. Patients were identified and patient data was obtained through our institutional Multidisciplinary Tumor Board (MDTB) database. Patients were classified by insurance as self-pay, public (Medicaid only and Medicare only), or commercial based upon insurance at diagnosis. TNM staging was described using the National Comprehensive Cancer Network staging guidelines; high stage was defined as stage IIb or greater for colorectal cancer and as stage IIIa or greater for anal cancer. Our study found a significant difference in patients covered under public insurance presenting with high stage cancer compared to patients covered under commercial insurance (P<.05), with 84% of patients with public insurance and 61% of patients with commercial insurance presenting with high stage cancer. There was a significant difference in insurance classification with respect to race (P<.05), with more non-white patients utilizing self-pay and public insurance as payment. While no significant difference in high stage at diagnosis was found with respect to race, 26% of non-white patients presented with stage IV cancer compared to 14% of white patients. These findings suggest the contribution of demographic and health insurance status to disparities in colon, rectal, and anal cancer patients, and may be attributed to lack of access to health care and effective cancer screening.
SEDATION IN THE BURN INTENSIVE CARE UNIT: A COMPARISON OF PROTOCOLS OVER TIME. Ryan Miller, Sponsored by Kaitlin McGinn, PharmD, Steven Kahn, MD, Sidney Brevard, MD, Linda Ding, MD, Jon Simmons, MD. University of South Alabama Medical Center, Department of Surgery, Division of Acute Care Surgery and Burns.

Continuous sedation is a commonplace treatment in an ICU setting. However, the use of prolonged sedation is associated with increased ICU length of stay (LOS), as well as duration of mechanical ventilation and ICU delirium in critically ill adults. Although these outcomes have been studied in mixed ICU populations, there is little published data in critically-ill burn patients. Based on the published literature, the University of South Alabama Burn Team implemented a protocol of spontaneous awakening and breathing trials (SAT/SBT) in January of 2012. A second protocol was added in August of 2015, mandating lighter levels of sedation and minimal use of benzodiazepine sedatives. This study seeks to longitudinally compare clinical outcomes in critically ill burn patients before and after the implementation of each protocol. It also seeks to evaluate the changes in clinical practice that have become more conservative towards mechanically ventilating patients with large burns. We hypothesize that clinical outcomes have improved with treatment advances and protocol implementation in the Burn ICU. This study was conducted as a single center, retrospective, observational comparison. Patients ≥19 years of age with ≥20% Total Body Surface Area (TBSA) burns were included. All patients who expired within 48 hours of admission were excluded. Sedation levels were measured using the Richmond Agitation Sedation Scale (RASS) and delirium screening was conducted using the Confusion Assessment Method-ICU (CAM-ICU). Study groups had similar demographics and TBSA. There was a clinically significant decrease in mortality (23.7% to 17.2% to 13.3%, p=0.3263). However, there was a clinically significant increase in hospital days (20 to 17.5 to 32.5, p=0.0722). Patients in the post-protocol groups showed a significant increase in ventilator-free days (11.5 to 25.5 to 25, p=0.0295), a decrease in days on the ventilator (13 to 2 to 3, p=0.0085), and spent more time within RASS goal (0 to 1, p=0.0385). This study supports that outcomes have improved with protocol implementation and as care is refined. Data collection should continue in order to gauge the long term effects of continued adherence to sedation protocols.
ABSTRACT

HERPES SIMPLEX VIRUS IN BURN PATIENTS. Kelsea Wright. Sponsored by Steven Kahn, MD and Kaitlin McGinn, PharmD, Department of Acute Care Surgery, University of South Alabama Medical Center, Mobile, AL.

Herpes simplex virus (HSV) is common in the human population and reactivation of a latent infection can occur during periods of stress or illness, such as a serious burn. Previous studies have shown that HSV infections may complicate the healing process and increase morbidity in burn patients. This study aimed to characterize the incidence of HSV burn wound infections among high risk patients at the University of South Alabama Medical Center (USAMC) and to describe the associated morbidity/mortality of infected patients. We conducted a retrospective chart review of 38 burn patients who had HSV titers drawn between September 2015 and April 2017. Outcomes were compared between patients with HSV titers who developed active infection (N=19) and those who did not (N=19). Patients identified with active infection and ≥20%TBSA and/or facial burns (N=17) were also compared to all patients with ≥20%TBSA and/or facial burns (N=60) during the study time period, regardless of titer status. Median length of hospital stay was 35 [9-62] days in patients with active HSV infection, compared to 12.5 [3-22.75] days in those with ≥20%TBSA and/or facial burns but no infection (P=0.007). Of patients with ≥20%TBSA and/or facial burns who were mechanically ventilated, median ventilator days were 22.5 [5.5-30.25] and 1.5 [1-4.5] for the active infection group (N=8) and the no infection group (N=10), respectively (P=0.023). Median ICU days of patients with ≥20%TBSA and/or facial burns were 36 (16.75-56.25) and 13.5 (12-22.5) for the active infection group (N=12) and the no infection group (N=16), respectively (P=0.01). In the active infection group, median HSV-1,2 IgM titers were 0.85 [0.55-1.16) in the active infection group, compared to 0.57 [0.43-0.79] in the no infection group (P=0.013). Of the 19 patients who had an active HSV infection, 17 (89.5%) had facial burns, suggesting that facial burns are a risk factor for developing an active infection. Active HSV infection is associated with unfavorable patient outcomes, and the risk of developing active infection should be especially considered in patients suffering facial burns.
ABSTRACT

PARENTING INTERACTIONS DURING PLAY BETWEEN MOTHERS AND CHILDREN IN THE GULF COAST REGION OF THE US TO SUPPORT POSITIVE DEVELOPMENTAL OUTCOMES. Aubrey Young. Sponsored by Elizabeth Kennedy, PT, PhD, PSC, and Stephanie Anderson, MD, Division of Developmental and Behavioral Pediatrics, Strada Patient Care Center, University of South Alabama College of Medicine, Mobile, AL.

Research has shown that the parent-child interactions during play can positively or negatively affect a child’s motor, cognitive, and social development. Studies have identified mother-child interactions as a consistent predictor of a child’s cognitive and social development. The purposes of this pilot study were to explore the feasibility of using the Parenting Interactions with Children: Checklist of Observations Linked to Outcomes (PICCOLO) within a High-Risk Infant Follow-Up clinic and compare the data obtained to established norms. PICCOLO is a strengths-based tool that identifies positive parenting interactions in four domains: affection, responsiveness, encouragement, and teaching. Although it has typically been used during home visits, PICCOLO has the potential to be a useful tool in clinical settings, to guide interventions and optimize patient outcomes.

PICCOLO was administered at the High-Risk Infant Developmental Follow-Up Clinic during appointments scheduled for Developmental monitoring, according to established protocol. Children followed in this clinic either have, or are at-risk for, developmental delays. Parameters for participation in this study were (1) the child must be between 10 and 47 months of age, (2) the mother must be present, and (3) the child must not have severe, uncorrected visual or hearing impairments. Half of our participants were African-American and the other half was Caucasian. Rater reliability was established through training videos and scoring comparison to maintain an inter-rater reliability of r=.80 as outlined by Roggman, et al. The researcher observed a 10-minute play interaction between mother and child and scored the PICCOLO during this interval. Parents were then counseled on the results of the PICCOLO, with emphasis placed on areas of strength.

Total administration time- including time required to obtain consent, observe play, and time spent counseling parents- was recorded. An anonymous, two-item satisfaction survey was given to all participants, as parent perception of PICCOLO is integral in determining its utility for clinical use.

We found that PICCOLO could be administered in an average of 12.4 minutes and did not extend the length of appointments. Furthermore, satisfaction was found to be high. In this limited sample, we found that our population’s scores did not vary significantly from established normative data except in the domain of Teaching. We determined PICCOLO to be feasible to implement in a clinic setting, providing a tool to nurture positive parenting interactions without placing undue burden on clinicians or families.
Due to the absence of functional uricase, humans have significantly higher uric acid concentrations than other species. Humans utilize free reactive oxygen species (ROS) in a nonenzymatic reaction to convert uric acid into allantoin, which provides a significant antioxidant buffer in human blood. While beneficial in some biological aspects, the buffer may also suppress the innate immune response causing increased susceptibility to sepsis in humans. We hypothesize that the elevated levels of uric acid in humans scavenges free ROS released by neutrophils thereby decreasing the efficacy of ROS-mediated bacterial killing. E.coli K-12 was challenged with hydrogen peroxide or Fenton’s Reagent (FR; 0.032 µg/µL hydrogen peroxide/0.0095 µg/µL Fe²⁺), which forms hydroxyl radicals, to imitate active ROS. Varying concentrations of uric acid were added to both the hydrogen peroxide and FR challenge to determine if there was a significant decrease in bacterial killing. E.coli growth in the presence of either hydrogen peroxide or FR was significantly reduced and uric acid (50µM) alone did not show significant toxicity. E.coli growth in a combination of hydrogen peroxide and uric acid did not show any significant change compared to the hydrogen peroxide challenge alone. However, E.coli growth in a combination of FR and uric acid (50µM) demonstrated 2.8-fold increased growth than in the FR challenge alone. These results support the hypothesis that uric acid scavenges free ROS released into the serum leading to a decrease in ROS-mediated bacterial killing.
ABSTRACT

COLORECTAL CANCER DETECTION BY EXCITATION-SCANNING HYPERSPECTRAL IMAGING. Will Martin. Sponsored by Silas Leavesley, Ph.D., Departments of Chemical and Biomolecular Engineering and Pharmacology, Center for Lung Biology, University of South Alabama College of Medicine, Mobile, AL.

Hyperspectral imaging has been implemented in biomedical applications such as the detection of molecular events and improved classification of cells, tissue, and biological components. Although the technology has great potential in the field of biomedical sciences its utilization has been limited in the clinical setting. This limitation is hallmarked by the complexity of the technology and the time required for acquisition of a single spectral image. My lab has demonstrated increased signal strength and faster acquisition times per sample through the use of the fluorescence excitation-scanning approach rather than the traditional emission-scanning approach. The excitation-scanning technique has been able to better delineate the spectral differences between colonic adenocarcinoma and normal colonic mucosa in flash-frozen tissues. In this presentation, I am reporting results that support the technique of excitation-scanning can be used to reliably screen pairs of fresh lesional and benign colorectal tissues. Using a novel hyperspectral imaging fluorescence excitation-scanning microscope, our colorectal tissues were excited by a range of wavelengths between 360-550 nm, at 5 nm increments. The resulting fluorescent emission above 550 nm was collected and the image data was corrected to achieve a NIST-traceable flat spectral response. An analysis of the data was performed via a range of supervised and unsupervised classification approaches within an imaging software- ENVI (Harris Geospatial Solutions). The supervised classification (Maximum Likelihood Analysis) resulted in >99% accuracy in a solitary patient image set, but a drop to 64% for multi-patient classification (n=9). We believe this drop can be attributed to an increase in false-positive detection rates and high inter-patient variability in cancer spectra. In conclusion, initial data indicates that this approach is a viable option in the detection of colorectal cancer. In the future, an increased patient population will need to be evaluated and the effects of inter-patient variability further investigated.
CONTROL OF BREAST CANCER CELL MIGRATION BY PROTEIN KINASES. Daniel Zieman. Sponsored by Lawrence LeClaire, Ph.D., Department of Biochemistry and Molecular Biology, University of South Alabama College of Medicine, Mobile, AL.

From embryonic development to wound healing, cell migration has been well established as an important biological process. In normal physiologic states, diverse types of motility and cell movement are driven by the actin cytoskeleton. However, the actin cytoskeleton also plays a key role in cancer cell invasion and metastasis. The transformation from normal physiology to cancerous pathology is facilitated by dysregulated protein kinases. In our laboratory, we have identified a protein called Nck-interacting kinase (NIK) that shows increased expression and activity in breast cancer cell lines. The increased activity of NIK has been shown to increase cell migration and invasion in multiple breast cancer cell lines. Previous studies of NIK have determined this positive correlation primarily by comparing the effect of EGF stimulation on normal MDA-MB-237 cells and those expressing dominant negative NIK. In pursuit of further evidence of NIK's role in breast cancer cell metastasis, we then questioned whether completely removing NIK from the cells would similarly impede the cells' ability to invade and migrate. By transfecting breast cancer cells with a plasmid encoding for a particular CRISPR/Cas9 protein complex directed toward the NIK gene, we were able to introduce mutations that effectively suppressed expression of functional NIK. PCR and Western blotting were used to provide evidence that NIK had been successfully knocked out. Our findings reveal that NIK can be removed from breast cancer cells using the CRISPR/Cas9 gene editing system, and further experimentation can be performed to determine its effect on breast cancer cell metastasis.
Abstract

THE SIGNIFICANCE OF A “B BUMP” ON M-MODE ECHOCARDIOGRAPHY AND ITS RELATIONSHIP TO SYSTOLIC OR DIASTOLIC HEART FAILURE AND NATRIURETIC PEPTIDE LEVELS. Grady Edge. Sponsored by Christopher Malozzi, D.O. and Bassam Omar, Ph.D., Department of Cardiology, University of South Alabama Medical Center, Mobile, AL.

The “B bump” has been identified in patients with elevated Left Ventricular End Diastolic Pressure (LVEDP) by using M-mode echocardiography. LVEDP signals a failing left ventricle or uncompensated congestive heart failure (CHF). The current study was conducted to assess the relationship of a “B bump” with the type of CHF and Brain Natriuretic Peptide (BNP) levels. Patient records and echocardiograms were obtained through Sorian and Syngo Dynamics software from the University of South Alabama Medical Center. BNP measurements were then obtained from the patient’s test nearest the echocardiogram along with degree of systolic or diastolic dysfunction recorded. These patients were compared to patients with severe systolic dysfunction whom did not present with a “B bump” and with patients with normal systolic function lacking a “B bump.” In general BNP levels were significantly more elevated in patients with a “B bump” (p=.046). Patients with reduced ejection fraction (EF<50%) with a “B bump” showed significantly elevated BNP levels compared to the reduced EF control group (p=.025). We conclude that “B bump” presentation in patients with systolic dysfunction correlates with a more severe BNP elevation.
How does Vitamin B3 Supplementation Effect Fructose Metabolism in Hepatocytes? Ellen Zhou. Sponsored by Marie Migaud, Ph.D., Department of Oncologic Science, Mitchell Cancer Institute, University of South Alabama College of Medicine, Mobile, AL.

Fructose bypasses phosphofructokinase, a major negative regulating enzyme in hexose metabolism. High fructose intake can result enzyme saturation and accumulation of metabolism that can alter other cellular enzymatic functions. HeLa cells were seeded in 6 well plates with DMEM, and incubated for 24 hours. These cells were then dosed with various NAD+ precursor with or without exposed to fructose. Cell counts were completed 24 hours after dosing. Comparing cells exposed to fructose with or without supplementation of NAD+ precursors, cells incubated with NAD+ precursors in addition to fructose exhibited higher grow rate. Comparing cells supplemented with NAD+ precursors with or without exposure to fructose, cells that were not exposed to fructose exhibited high growth and survival. Exposure to fructose negatively impacts cell growth and survival, but supplementation of certain NAD+ precursors can result in improvements.
ABSTRACT

RAD18 AND ITS CRUCIAL ROLE IN DNA DAMAGE REPAIR AFTER GLIOBLASTOMA CELL TREATMENT WITH TEMOZOLOMIDE. William Nicolson. Sponsored by Dr. Komaraiah Palle, Ph.D., Department of Oncologic Sciences, Mitchell Cancer Institute, University of South Alabama College of Medicine, Mobile, AL.

New cancer treatment options such as targeted therapies (hormonal treatment in breast cancer) have been recently developed, and they show promising results. However, brain cancer lacks any striking advances in therapeutic strategies in ways that other cancers have. Malignant Gliomas occur more frequently than any other type of central nervous system tumor. Even with aggressive treatment using surgery, radiation, and chemotherapy, median reported survival is less than 1 year. Temozolomide (TMZ), a new drug, has shown promise in treating malignant gliomas and other difficult-to-treat tumors. Temozolomide’s cytotoxicity to malignant cells is thought to occur through the alkylation of DNA. However, cancer cells exposed to temozolomide activate various repair mechanisms which in turn actively repair TMZ induced DNA damage, which ultimately results in TMZ resistance and disease recurrence. Rad18 plays a critical role in DNA repair. Rad18 is an E3 ubiquitin ligase recruited to sites of DNA damage. Along with the E2 ubiquitin ligase Rad6, Rad18 is responsible for monoubiquitination of DNA damage proteins including the replication clamp PCNA and the Fanconi anemia core protein FANCD2. Monoubiquitination of these proteins signals to downstream effector molecules and results in the repair of either post-replication repair lesions via the translesion synthesis (TLS) pathway or DNA double strand breaks via homologous recombination. Our results show that expression of Rad18 is elevated in different glioblastoma cell lines compared to the normal astrocytes. Furthermore, analysis revealed that TMZ treatment increases the expression of Rad18. Convincingly, our preliminary data shows that RAD18 is overexpressed in the various glioblastoma cells we have in our lab compared to normal astrocytes. U251 glioblastoma cells that are exposed to 100µM TMZ showed time dependent increase in RAD18 expression. RAD18’s importance has been highlighted through a decrease in monoubiquitinated FANCD2 in RAD18 inhibited U251 cells. Finally, TMZ treatment in Rad18 inhibited U251 cells showed increased DNA damage (γH2AX) compared to control cells.
ABSTRACT

SENSITIVITY OF MELANOMA CELLS TO A NOVEL CLASS OF RAS INHIBITORS. Davis Diamond. Sponsored by Gary Piazza, Ph.D., Drug Discovery Research Center, Mitchell Cancer Institute, University of South Alabama, Mobile, AL.

A high percentage of human cancers originate from gain of function mutations in the ras gene, locking the protein in a constitutively active GTP-bound state that promotes tumor cell proliferation, survival and metastasis. In melanoma specifically, 15-20% of all cases are caused by ras gene mutations, resulting in a constitutively active N-Ras protein. Mutations in the ras gene or constitutive activation of upstream tyrosine kinase receptors drive tumor cell growth by activation of Raf/MAPK and PI3K/AKT signaling pathways. Ras has been a long sought-after target for cancer treatment, yet it has remained elusive. Currently, there are no inhibitors available to treat Ras-driven cancers. Screening a library of indene derivatives in a differential phenotypic assay identified a novel compound class displaying high potency and selectivity to inhibit the growth of tumor cells harboring mutant Ras relative to tumor cells with wild type (WT) Ras. Lead optimization resulted in a drug development candidate, ADT-007, with an IC50 value in the low nanomolar range and selectivity indices of 100 fold or greater to inhibit the growth of tumor cells with constitutively activated Ras relative to tumor cells with low levels of activated Ras. Sensitivity among a large panel of tumor cell lines to this compound class strongly correlated with levels of activated Ras, but did not appear to be limited to a specific ras gene mutation or Ras protein isoform. The Drug Discovery Research Center at Mitchell Cancer institute has reported that ADT-007 potently and selectively inhibit the growth of the human melanoma cell line, SK-MEL-2, harboring a mutation in the ras gene that encodes the constitutively active N-Ras protein with an IC50 value of 7 nM. In addition, both compounds potently inhibited the growth of the murine melanoma cell line(s), B16-F10 and B16-BL6, with WT ras, but harboring a mutation in a tyrosine kinase receptor upstream of Ras, specifically PDGFRα, which results in high levels of active, GTP-bound Ras as confirmed by Ras-RBD pull-down assays. To determine if treatment disrupts Ras signaling, B16-F10 and B16-BL6 cells were incubated with the compound before being subjected to Western blotting for phosphorylated signaling molecules downstream of Ras. Treatment shows reduced levels of phosphorylated c-Raf, MEK, and ERK at concentrations that inhibit tumor cell growth.
A POPULATION HEALTH STUDY: EVALUATING QUALITY OF LIFE IN TYPE-II DIABETIC PATIENTS  Malik J. McMullin, M2, Sponsored by Barry E. Porter, Ph.D., Carol P. Motley, MD, Department of Family Medicine, University of South Alabama Health System, Mobile, AL.

The healthcare system in the United States is suffering a major crisis. As many reforms are occurring in the everchanging field of healthcare policy, patients continue to suffer by experiencing inconsistent care. Compounding that issue, social determinants of health further exacerbate the diseases experienced by complex patients. Population Health is comprised of a wide range of interdisciplinary care models that describes a multi-initiative approach to meet the needs of and lower the cost for systems to provide complex patients with optimized care. This study was a pilot for an instrument to be used for measuring patient Quality of Life called PROMIS (Patient-Reported Outcomes Measurement Information System), a scale validated by the National Institutes of Health. Convenience sampling was used for this study; and the first sixty-two patients with prediabetes or diabetes, who agreed to participate in the practice research, were administered the PROMIS-29. These patients had a diagnosis of prediabetes or diabetes (Type II) in their medical record with a hemoglobin A1c of 5.8 or higher from their last visit. In addition to the PROMIS, we separated patients into four groups based on their A1c levels. These groups are as follows: Group A, Prediabetic (5.8 - 6.5); Group B, Well-Controlled (6.6 - 8.0); Group C, Moderately-Controlled (8.1-10.0); Group D, Inadequate Control (10.1 - Higher). The total sample size for this study is n=62. The groups within the sample are distributed as follows: Group A (n=18), Group B (n=21), Group C (n=16), and Group D (n=7). The PROMIS-29 measures eight domains in QoL. They are as follows, ranked highest to lowest overall: Pain Interference (µ= 58.11), Fatigue (µ= 53.51), Sleep Disturbance (µ= 53.180), Anxiety (µ= 52.92), Depression (µ= 50.83), Ability to Participate in Social Roles and Activities (µ= 49.80), Physical Function (µ=42.14), and Pain Intensity (µ= 4.95). Each domain of the PROMIS-29 is scored by participants using a Likert rating scale from 0-5. These ratings are converted into t-scores using a scoring algorithm provided by Health Measures, the developer of the instrument. The t-scores are then typically used on an individual level to determine a QoL score that allows healthcare providers to gauge what areas may need attention in either physical health, mental health, or social health. Pain intensity is evaluated using a Likert rating scale 0-10 causing it to be the lowest overall. Use of the PROMIS-29 for practice research to improve the quality of care revealed positive results in its applicability, data quality, and potential for assessing patient QoL throughout the care process.
ABSTRACT

CLONING, EXPRESSION, AND PURIFICATION OF UBE3B FOR DOWNSTREAM CRYSTALLIZATION
Chase Diard, Brandon D'Arcy, Robert W. Sobol, Aishwarya Prakash
Mitchell Cancer Institute, University of South Alabama
1660 Springhill Ave, Mobile, AL

Ubitquitylation is an extremely important cellular process that involves tagging molecules with ubiquitin for eventual degradation by the 26S proteasome. The human enzyme UBE3B is a calmodulin-regulated, mitochondria-associated, E3 ubiquitin ligase. Mutations in this enzyme can lead to an autosomal recessive disease known as Kaufman oculocerebrofacial syndrome, with symptoms such as severe intellectual disability, distinctive craniofacial features, and a wide variety of eye problems. UBE3B has been previously determined to be difficult to study structurally owing to its size and regions of disorder. To overcome this, we cloned 4 constructs of UBE3B: the IQ domain, the HECT domain, the substrate binding domain with the IQ domain, and the substrate binding domain without the IQ domain. Each subunit was Gateway cloned into the vector pDEST-His-Mbp. After cloning, Rosetta2 E. coli cells were transformed and expression was induced with IPTG. Expression was tested by lysing the cells followed by gel electrophoresis. Purification was attempted using metal affinity chromatography. After cleavage of the His-tag and MBP-fusion protein using TEV protease, subtractive chromatography was used to isolate the protein of interest. Currently, all 4 constructs have been cloned into destination vectors and expression and purification of the IQ domain was performed.
ABSTRACT

THE CORRELATION BETWEEN SERUM MAGNESIUM LEVEL AND TROPONIN I LEVEL ELEVATION IN PATIENTS ADMITTED WITH ACUTE CORONARY SYNDROME. Hunter Childers. Sponsored by Bassam Omar, M.D., Ph.D., Department of Cardiology, University of South Alabama Medical Center, Mobile, AL.

Low levels of serum magnesium can contribute to the incidence of arrhythmias. Given that arrhythmias are of particular concern following acute coronary syndrome (ACS), it was once common practice to administer magnesium to patients that present with ACS in order to help prevent damage associated with arrhythmias occurring in an ischemic heart. This was justified by studies that suggested mortality improved in patients receiving intravenous magnesium (Rasmussen et al.). However, the study “Early administration of intravenous magnesium to high-risk patients with acute myocardial infarction in the Magnesium in Coronaries (MAGIC) Trial: a randomized controlled trial” showed no measurable improvement in short term outcomes between patients receiving supplemental magnesium on admission and those who don’t (Antman et al).

Our study focuses on the association between serum magnesium lower than 1.8 mg/dL on admission and peak troponin I levels compared to peak troponin I in patients with serum magnesium levels on admission over 1.8 mg/dL. Our comparison found that patients with a low serum magnesium level had a mean peak troponin of 25.9 while those with a serum magnesium over 1.8 mg/dL had a mean peak troponin of 16.8 with a p value of 0.022. Given that patients who present with normal or higher serum magnesium levels have lower peak troponin I levels further studies may suggest that magnesium supplementation in patients with coronary artery disease could lessen the damage caused by an infarction if one occurs.
ABSTRACT

ANALYSIS OF MATRIX METALLOPROTEINASES EXPRESSION IN MOUSE MAMMARY TUMORS WITH IMPROVED MITOCHONDRIAL DNA REPAIR. Benjamin Gibson, Larysa Yuzefovych, Mita Patel, Viktor Pastukh. Sponsored by Lyudmila Rachek, Ph.D., Department of Pharmacology, University of South Alabama College of Medicine, Mobile, AL.

Introduction: Using a genetic model of breast cancer (PyMT mouse), we have previously obtained proof-of-concept results that mitochondrial DNA (mtDNA) damage plays a critical role in oxidative stress and breast cancer progression. We found that targeting of the DNA repair enzyme hOGG1 to mitochondria significantly reduced mammary tumor incidence and metastasis in double transgenic PyMT/MTS-hOGG1 mice. Additionally, we have utilized a translational strategy of targeting the recombinant DNA repair enzymes into mitochondria of breast cancer cells using a TAT-protein transduction system. However, the exact mechanisms by which mtDNA repair protects against breast cancer progression need to be defined. Increased expression of matrix metalloproteinases (MMPs) has been associated with the invasion and metastasis of malignant tumors of different histogenetic origins. The goal of this study was to determine if preventing mtDNA damage, by targeting DNA repair enzymes into mitochondria using genetic models or TAT-protein transduction, will reduce the expression of MMP-2 and MMP-9 in mammary tumors and thereby limit breast cancer progression and metastasis.

Methods: We performed analysis of mRNA and protein levels using qRT-PCR and Western blot, respectively. Mitochondrial morphology in primary tumors cells was evaluated using microscopy with fluorescent mitochondrial probes.

Results: Gene expression analysis showed that preventing mtDNA damage by targeting DNA repair enzymes into mitochondria using genetic models reduced MMP-2 expression on both mRNA and protein levels. Patterns of MMP-9 expression showed that preventing mtDNA damage reduced protein expression level. No consistent pattern of MMP-9 expression was observed in RNA isolated from tumors with altered mtDNA repair. Additionally, primary tumor cells isolated from PyMT/OGG1-/- and PyMT/OGG1-/-/MTS-hOGG1 mice showed increased cell and mitochondrial size when compared to primary tumor cells isolated from PyMT/wild type mice.

Conclusions and future directions: Our findings suggest that both MMP-2 and MMP-9 protein expression patterns are associated with mtDNA damage, oxidative stress, tumor progression, and metastasis. Additional studies are in progress to further characterize the role of mtDNA damage in the gene expression of MMPs and other breast cancer markers of epithelial-to-mesenchymal transition. Also, future studies will be performed to evaluate the role of mtDNA damage in mitochondrial morphology.
ABSTRACT

COMPARATIVE ANALYSIS OF NISSEN FUNDOPLICATION AND MAGNETIC SPHINCTER AUGMENTATION FOR THE TREATMENT OF MEDICALLY REFRACTORY GERD. Carly McRae. Sponsored by William Richards, MD., Department of Surgery, University of South Alabama College of Medicine, Mobile, AL.

Some patients experience persistent symptoms of Gastroesophageal Reflux Disease (GERD) despite maximization of Proton Pump Inhibitor (PPI) medication. Magnetic Sphincter Augmentation (MSA) is a novel surgical approach to the treatment of severe GERD, in which magnetic beads are secured around the lower esophageal sphincter, augmenting the LES function as an anti-reflux barrier. We hypothesize that patients undergoing MSA will achieve GERD relief, equal to that obtained after Laparoscopic Nissen fundoplication (LNF). The GERD Health Related Quality of Life Questionnaire (GERD HRQL) is a validated clinical tool that was used to quantify patient outcomes on PPIs and after ARS. We participate in a multicenter prospective IRB approved database “Registry Outcomes Anti-Reflux Surgery” that applies objective and subjective information about patients undergoing anti-reflux surgery. Information from the database and HRQL scores were used to compare the effectiveness of medical intervention with ARS (LNF and MSA). Results are expressed as a Mean± SEM, and single-factor AVOVA was used to compare groups.

The data indicates that compared to medical intervention (HRQL = 23.5 ± 13.9), ARS demonstrates a significant decrease (P<.05*) in the GERD associated symptoms. There was no significant difference in the GERD HRQL between LNF and MSA. Pre and post-operative GERD HRQL surveys completed by patients participating in our study indicate that compared to medical treatment alone (7% satisfied), surgical intervention for the treatment of GERD greatly increases patient satisfaction in regards to reflux symptoms. LNF was shown to lead to 83% patient satisfaction, while MSA produced 87% of patients who were either Satisfied or Neutral with their outcomes and 13% of patients who were dissatisfied. 97% of patients receiving medical intervention alone for the treatment of GERD reported being able to belch, with 3% reporting that they were unsure of their ability to belch. 100% of patients undergo LNF reported being able to belch while 95% who underwent MSA reported being able to belch and 5% reported being unsure of their ability to belch. The data collected in the questionnaires suggests that both the LNF and MSA, are far more effective at reducing GERD symptoms than PPI use in this group of patients. The data also suggests that both LNF and MSA lead to similar patient outcomes in terms of both patient satisfaction and ability to belch post-operatively.
ABSTRACT

Recovery from Acidification in Pulmonary Artery and Pulmonary Microvascular Endothelial Cells Reveals the Activity of an Amiloride-insensitive Sodium Dependent Transporter. Dylan Adams. Sponsored by Sarah Sayner, Ph.D., Department of Physiology and Cell Biology, Center for Lung Biology, University of South Alabama College of Medicine, Mobile, AL.

RATIONALE: Increased pulmonary endothelial permeability is a life-threatening complication associated with acute respiratory distress syndrome (ARDS). These patients often require ventilatory support; however, the protective mechanical ventilation strategy employed results in hypercapnic acidosis. The acidosis is often corrected with sodium bicarbonate infusion which can exacerbate ARDS. Our recent studies reveal that bicarbonate increases pulmonary endothelial permeability, and lipopolysaccharide (LPS)-induced increase in pulmonary endothelial permeability is dependent upon stimulation of soluble adenylyl cyclase isoform 10 (AC10, a.k.a. sAC). While pulmonary microvascular endothelial cells (PMVECs) and pulmonary artery endothelial cells (PAECs) both express AC10, only PMVECs have a bicarbonate stimulated cAMP pool, which is dependent upon sodium, suggesting a role for Na⁺-bicarbonate cotransporters (NBCs). The activity of NBCs can be determined by recovery from cellular acidification, but the role of Na⁺/H⁺ exchangers (NHE) also needs to be considered. The goal of these studies was to examine the difference in recovery from cellular acidification between PMVECs versus PAECs and PMVECs in which NBCn2 isoform had been partially knocked down versus control cells. The studies were conducted in a bicarbonate/CO₂ free environment to isolate NHE activity. METHODS: The ammonium prepulse technique was used to acidify the cells and the ratiometric dye, BCECF-AM, was used to detect intracellular pH fluctuations via fluorescence microscopy. The recovery from acidification was recorded in the presence and absence of both sodium and amiloride (an inhibitor of NHE). RESULTS: Following ammonium prepulse washout, the acidification of all the cells was similar, and there was minimal recovery from acidification in the presence of amiloride and absence of sodium. When sodium is added back to the buffer, the rate of recovery from acidification was more rapid in PAECs versus PMVECs. In PMVECs that were engineered to knockdown NBCn2, the rate of recovery was similar to the scrambled control. When amiloride was removed from the buffer, the final recovery from acidification was higher in PAECs versus PMVECs either with or without NBCn2. CONCLUSION: In pulmonary endothelial cells, there appears to be an amiloride-insensitive, sodium dependent recovery from acidification that is more prominent in PAECs versus PMVECs. This recovery from acidification suggests the expression of NHE4, an amiloride-insensitive isoform, in pulmonary endothelial cells.
ABSTRACT

OVERUTILIZATION OF HELICOPTER EMS IN THE CENTRAL GULF COAST REGION: A SINGLE CENTER RETROSPECTIVE COHORT STUDY. Justin Beasley. Sponsored by Jon D. Simmons MD, Department of Surgery, Division of Trauma and Surgical Critical Care, University of South Alabama Medical Center, Mobile, AL.

The use of air transport for trauma has increased significantly over the past decade. This is likely attributed to the presumption of decreased transit times compared to their counterparts on the ground; however, given its much higher cost, recent studies have called into question the true benefit and cost-effectiveness of helicopter EMS (HEMS). In Alabama, the decision to use HEMS instead of ground ambulance is made by EMT's on scene using guidance from the Alabama Department of Public Health EMS protocol and the Alabama Trauma Communications Center (ATCC). This protocol allows for entry into ATCC by anatomic, physiologic, and mechanism criteria. In order to determine the current utilization of HEMS in our catchment area and the accuracy with which on-scene triage decisions were made, we queried the USA trauma registry and ATCC database for all adult trauma patients transported via helicopter to the Medical Center from January 2015 through April 2017. The patients were grouped by the triage decision as being appropriate (i.e. admission to ICU, required emergent operation, or death) or inappropriate (i.e. admission to the ward or discharged home without admission). During the study period, 380 patients were identified. 247 (66.3%) and 133 (33.7) were designated as appropriate and inappropriate, respectively. Over 7% of the entire cohort was discharged home without admission following HEMS. Also, only 73% of patients in the appropriate group and 44% of patients in the inappropriate group had one or more criteria for ATCC referral. Only a few of the ATCC criteria were statistically different between groups but none were predictive of appropriateness of HEMS use. Further complex statistical modelling in larger cohorts may determine if a specific combination of criteria are predictive. In conclusion, these findings suggest that current triage criteria for helicopter transport and entrance criteria for the ATCC should be reevaluated to reduce over-triage and unnecessary healthcare costs.
ABSTRACT

TUMOR INTRINSIC B7-H3 (CD276) MODULATES TUMORIGENIC PROPERTIES OF OVARIAN CANCER CELLS. Taylor Young¹, Alla Musyienko¹, Holly Taylor¹, Elaine Gavin¹, Ileana Aragon¹, Jennifer Scalici¹, Rodney Rocconi¹, Luciana Madeira da Silva¹. ¹ Gynecologic Oncology Research Lab, Mitchell Cancer Institute, University of South Alabama College of Medicine, Mobile, AL.

Ovarian cancer is the leading cause of death from gynecological malignancies. B7-H3 (CD 276) is a cell surface glycoprotein that has been considered a valuable novel biomarker and a promising target for cancer treatment. B7-H3 is overexpressed in a wide variety of human cancers, including ovarian and endometrial cancers, and is associated with tumor progression, metastasis, and poor patient outcome. In addition to its function in cancer immunity, B7-H3 also has tumor intrinsic functions related to drug resistance, metabolic reprogramming, migration and invasion of cancer cells. It has recently been reported that exogenous sB7-H3 promotes the invasion and metastasis of pancreatic cancer cells through the TLR4/NFkB pathway. We have detected soluble B7-H3 (sB7-H3) in blood serum and malignant ascites of ovarian cancer patients. B7-H3 overexpression in the ovarian tumors and tumor vasculature has been reported and B7-H3 monoclonal antibodies have shown inhibitory effects in ovarian cancer xenograft studies. Nonetheless, the molecular pathways regulated by B7-H3 in ovarian cancer remain largely unknown.

Here we explore the role of tumor intrinsic and soluble B7-H3 in ovarian cancer cells by two distinct approaches: 1) genetic modulation of B7-H3 expression levels in ovarian cancer cells using (a) CRISPR/Cas9-mediated B7-H3 knockout and (b) B7-H3 overexpression by a retroviral transduction system; and 2) recombinant human B7-H3 as a source of soluble B7-H3.

We aimed to investigate the role of B7-H3 in the migratory and invasive properties of ovarian cancer cells, as well as potential signaling pathways elicited by soluble B7-H3. Our laboratory had previously generated a B7-H3 knockout ovarian cancer cell line, OV-90 B7-H3KO, which showed remarkably decreased ability to establish tumors in immunocompromised mice when compared to B7-H3 wild-type (WT) control cells. We also had previously generated TOV112D B7-H3 overexpressing cells and we are currently evaluating their tumorigenicity in vivo. Using a Boyden chamber transwell assay to assess migration of cancer cells, here we show that knockout of B7-H3 results in defective migration of OV-90 ovarian cancer cells. Conversely, overexpression of B7-H3 increases migratory properties of TOV112D cells. However, adding recombinant B7-H3 up to a concentration of 10µg/ml even with a 48h pre-incubation of cells did not affect migration of TOV112D cells, a result that differs from literature on pancreatic cancer. Mechanistically, while our previous data showed that knockout of B7-H3 decreases STAT3 and NFkB signaling in ovarian cancer cells, recombinant B7-H3 did not. Altogether, our results revealed a role for tumor intrinsic but not recombinant B7-H3 mediating migration of ovarian cancer cells.
Pancreatic cancer is one of the most lethal malignancies in the United States. It currently is the fourth leading cause of cancer-related deaths, but by the year 2030, it is expected to become the second leading cause of cancer-related deaths. The poor outcome from pancreatic cancer can be attributed to late diagnosis of disease, a lack of effective therapy for treatment, and chemo-resistance. Therefore, identification of novel molecular targets and understanding the mechanisms underlying the aggressive nature of pancreatic cancer remains to be a prime focus area of research.

Epidemiological studies have affirmed the association between obesity and raised incidences of a variety of cancers including pancreatic cancer. Obesity is believed to be a major risk factor of pancreatic cancer; it has been showed that a BMI greater than 35 increases the relative risk by about 50%. Though, the exact mechanism by which obesity induces cancer is poorly understood, however, resistin is believed to be a connecting link between inflammatory processes induced by obesity and cancer development. Resistin, primarily expressed by mononuclear leukocytes, macrophages, and bone marrow cells in humans, is a cytokine known to have potent pro inflammatory properties. Accumulating data suggests that resistin not only serves as an inflammatory biomarker but also acts as a potential mediator in obesity-associated diseases, including cancer. Additionally, many studies have shown that resistin plays a role in several types of malignancies including breast cancer, prostate cancer, and colon cancer. Recently, it has been shown that patients diagnosed with pancreatic cancer have significantly higher serum levels of resistin than controls.

Here, we investigated the role of resistin in the pathobiology of pancreatic cancer. Our preliminary data shows high levels of resistin in the serum samples of pancreatic cancer patients as compared to healthy individuals. In addition, we observed resistin receptor, CAP1, is aberrantly expressed in a set of pancreatic cancer cell lines, but not resistin.

Furthermore, we demonstrated treatment that resistin treatment of CAP1 expressing pancreatic cells significantly enhanced growth, reduced the doubling time of the cells and aggressiveness as observed by cell migration and invasion assay. In all, the data collected thus far shows resistin plays an important role in the growth, aggressiveness, and stemness of pancreatic cancer cells.
ABSTRACT

LOSS OF PARP1 INVOLVED IN THE DNA DAMAGE RESPONSE (DDR) PROVIDES INCREASED CANCER CELL SENSITIVITY TO CHEMOTHERAPEUTICS AND RADIATION. Tanner McGill. Sponsored by Jennifer Clark, Ph.D., Peter Sykora, Ph.D., Joel Andrews, Ph.D., and Robert Sobol, Ph.D., Department of Oncological Sciences, Mitchell Cancer Institute, University of South Alabama College of Medicine, Mobile, AL.

The ability of cancer cells to repair DNA damage induced by chemotherapy and radiation contributes significantly to clinical resistance. PARP1, an enzyme that is involved in repairing DNA damage via Base Excision Repair, has shown promise as a target for cancer therapy. Inhibiting the involvement of PARP1 in the DNA repair process in cancer cells is known to increase the effectiveness of DNA damaging therapies. It is important to better understand this enzyme's activity in DNA damage and repair so as to further determine the best way to target this activity in cancer treatments. The goal of this project is to establish whether the loss of PARP1 causes an increase in the sensitivity of cancer cells to chemotherapeutic drugs and radiation. Gene editing via the CRISPR/Cas9 approach was used to cause a disruption to the PARP1 gene to create a knockout of PARP1. Following immunoblot confirmation that the PARP1 protein was not expressed in the cells, each was then exposed to H₂O₂ and MNNG (two different DNA damaging agents) and the amount of DNA damage was analyzed using the CometChip assay. Our results demonstrated that loss of PARP1 increased sensitivity to H₂O₂ (single stranded breaks and oxidative damage) but not MNNG (alkylating damage). Expression of fluorescent-tagged repair proteins (PolB and XRCC1) in these cells revealed a decreased recruitment of these proteins to sites of damaged DNA induced by laser microirradiation. These data further confirm what is known about PARP1 as well as the fact that after being knocked out, the cells become more sensitive to a multitude of facilitated damage. Future studies using this approach will include MTT assay, more damaging agents, and further manipulation in order to study the DNA Damage Response proteins more thoroughly.
Cancer mutations of the DNA repair gene DNA polymerase beta impact protein stability Matthew Kassels, Qingming Fang & Robert W. Sobol
Molecular and Metabolic Oncology Program, University of South Alabama
Mitchell Cancer Institute, Mobile, AL

DNA polymerase $\beta$ is an important enzyme in the process of base excision repair (BER) and functions by replacing the nucleotide once the damaged base is removed. Mutations in DNA polymerase $\beta$ (Pol$\beta$) can be detrimental to cell function, leading to unrepaired lesions and mutations that can ultimately lead to cancer. One specific mutation occurring in the V303 loop (TM) of DNA polymerase $\beta$ has been shown to lead to decreased ability to bind the scaffold protein XRCC1 (another critical BER protein). Our goal was to clone, express, and purify recombinant DNA polymerase $\beta$(TM) and then characterize this enzyme and its interaction with XRCC1. We first constructed plasmids for expression of GST-tagged-Pol$\beta$(TM) in E. coli by polymerase chain reaction (PCR) cloning. The fusion of the GST protein, which is a glutathione S transferase protein, to the N-terminus of Pol$\beta$, allows rapid and highly selective purification of the recombinant protein. The PCR products were purified and then enzymatically digested in order to ligate the recombinant DNA into the E. coli expression plasmid. Once ligated, the plasmids were transformed into E. coli cells and plated on LB agar (plus ampicillin) for isolation. The vectors were validated by DNA sequencing and were then selected to be used for protein expression and purification. The protein purification process began with sonication to lyse the E. coli cells. The E. coli lysates were then run through a glutathione resin column so that the GST-tagged-Pol$\beta$(TM) mutant protein binds to the column. A GST-Tev protease was then added to free the Pol$\beta$(TM) mutants for collection. The lysate was then run through a Mono-S cation exchange column and size exclusion column to isolate the purified Pol$\beta$(TM) protein. The TM mutants will be used at a later to test for activity and ubiquitiylation.
ABSTRACT

IS LACK OF EYE GAZE TOWARDS A CAMERA ASSOCIATED WITH ATTENTION DEFICIT/HYPERACTIVITY DISORDER (ADHD), ANXIETY, COGNITIVE OR LANGUAGE DELAYS, AND/OR SEVERITY OF SOCIAL DEFICITS IN CHILDREN WITH AUTISM SPECTRUM DISORDER (ASD)? Cade Loftin. Sponsored by Stephanie Anderson, MD, Kim Zlomke, Ph.D., and Hanes Swingle, MD, MPH. Department of Pediatrics, Division of Developmental and Behavioral Pediatrics, University of South Alabama.

PURPOSE: This study investigates whether failure to gaze at a camera during a clinic photograph is correlated with higher likelihood of social, cognitive, or language delays or with other problematic behaviors in children with ASD.

METHODS: A retrospective chart review was conducted. Children with ASD between 3 and 6 years of age who were not gazing at the camera in their clinic photographs were compared with children with ASD who were gazing at the camera. We compared the two groups across multiple domains of development and behavior, using the following:

- Behavior Assessment System for Children, 2nd or 3rd Edition (BASC-2 or BASC-3), Parent Rating Scales scores for Hyperactivity, Anxiety, Atypicality, and Withdrawal

We also compared the rates of gazing at the camera in neurotypical children without family history of ADHD or developmental delays to a random sample of patients on the autism spectrum. Statistical analysis was conducted using the chi-square tests and ANOVA.

RESULTS: Only 1 of the 21 (4.8%) neurotypical children failed to look at the camera, compared to 13 of 51 (25.5%) children diagnosed with ASD (p<.05). When we compared children with ASD who looked at the camera to those who did not, we identified no significant differences in standardized scores on the assessment tools examined in this study.

CONCLUSION: Children with ASD are more likely to be looking away from a camera compared to neurotypical children (p<.05) when their pictures are taken in a clinic setting. Failure to look at a camera in children with ASD was not statistically associated with measures of ADHD, anxiety, withdrawal, atypical behaviors, or measures of socialization on the ADOS-2.
MIR4728-MEDIATED NF-KB SIGNALING ACTIVATION VIA TRAF6 IN BREAST CANCER CELLS. Samanta Mukkamala. Sponsored by Ming Tan, M.D., Ph.D., Department of Oncological Sciences, Mitchell Cancer Center, University of South Alabama College of Medicine, Mobile, AL.

ErbB2-intronic microRNA-4728 is a known novel tumor suppressor and antagonist of oncogenic MAPK signaling through direct targeting of ERK upstream kinase MST4. miR-4728 exerts numerous tumor-suppressive properties and is under-expressed in breast tumors compared to normal tissue. Because miR-4728 suppresses ERK activation, we hypothesized that it would regulate a kinase that activates ERK via MST4. MST4 functions to promote cell proliferation and cell polarity and thus may aid in tumor growth. MST4 is also a negative regulator of inflammation through phosphorylation of the adaptor TRAF6. TRAF6 activation leads to downstream signaling activation of the transcription factor NF-κB and production of inflammatory cytokines. Because TRAF6 has been identified as an oncoprotein that promotes Ras-driven activation of NF-κB, its role in activation of NK cells is being explored, as NK cells release cytokines and chemokines that induce an inflammatory response to kill tumor cells. NK cells are activated by INF-γ, and we propose that overexpression of TRAF6 in MD-MBA 231 breast cancer cells will lead to increased NF-κB expression, which will result in upregulation of INF-γ in 231 cells. Therefore, results could indicate that overexpression of TRAF6 could be a potential therapeutic target in breast cancer through increased INF-γ signaling.
ABSTRACT

DEVELOPMENT AND CHARACTERIZATION OF NOVEL PPP-FAMILY INHIBITORS TO AID OUR UNDERSTANDING OF THE ROLE PLAYED BY SERINE/THREONINE PHOSPHATASE 5 (PP5C/PPP5C) IN GLUCOCORTICOID SIGNALING. Erin S. Bouska; Sponsored by Richard Honkanen, Ph.D., Department of Biochemistry and Molecular Biology, University of South Alabama College of Medicine, Mobile, AL.

Corticosteroid insensitivity creates a continual challenge to the wellbeing of patients with severe asthma, and thus represents a significant unmet medical need. Because of this, understanding the biology of glucocorticoid actions at a cellular level is crucial to the development of new treatment options. Serine/threonine protein phosphatase 5 (PP5C) is known to alter glucocorticoid receptor (GR) actions, and PPP5C overexpression has been implicated in the promotion of corticosteroid insensitivity in patients with severe asthma (Bouazza, B. et al 2012: Am J Respir Cell Mol Biol Vol 47, Iss. 4, pp 464–473). These studies suggest that changes in PPP5C activity within airway cells could affect corticosteroid responsiveness in patients with corticosteroid-insensitive asthma. However, the exact action of PP5C on the GR within the airways is currently unclear. Our hypothesis is that small molecule inhibitors of PP5C catalytic activity will produce an increase in the phosphorylation of GR at key serine residues, and in doing so alter the expression or actions of a limited subset of GR-responsive genes and proteins. To test this hypothesis, we need to first develop small molecule inhibitors of PP5C. Thus, we screened a library of >315,000 small molecules, identifying ~20 compounds that share a 7-oxabicyclo[2.2.1]heptane-2-carbonyl moiety. Here, eight novel compounds developed from this scaffold were tested for inhibitory activity against PP1C, PP2AC and PP5C using an established fluorescent intensity (FLINT) assay. These studies identified compound 67 as the most potent inhibitor of PP5C (PP5C; IC50 = 0.450 +/- 0.056 μM, n=4; PP1 IC50 = 2.16 +/- 0.13 μM, n=4). Compound 64 demonstrated less potency but greater selectivity (PP5: IC50 = 2.08 +/- 0.012 μM, n=4; PP1C, IC50 = 12.1 +/- 0.23 μM, n=4 respectively). To confirm the actions of Compound 67 in a biologically relevant setting, 67 was tested in a cell based assay to determine if it can mimic cellular responses produced by the genetic disruption of PPP5C expression. PPP5C gene disruption was achieved using the CRISPR-Cas 9 system in HEK-293 cells to produce a positive control (i.e. an inhibitor capable of completely inhibiting PP5C should mimic the affects produced by genetic deletion of PPP5C). In HEK-293 cells that do not express PPP5C, spontaneous activation of AKT1 is observed, which is measured using Western analysis and phosphorylation specific antibodies.
ABSTRACT

BICARBONATE IS REQUIRED FOR PROLIFERATION OF PULMONARY ENDOTHELIAL CELLS. Tyler Harvell. Sponsored by Sarah Sayner, Ph.D., Department of Physiology and Cell Biology, Center for Lung Biology, University of South Alabama College of Medicine, Mobile, AL.

RATIONALE: Adenylyl cyclase 10 (AC10 or sAC) is a soluble AC, which localizes to the cytosol. Although bicarbonate is traditionally regarded in regulation of pH, bicarbonate also stimulates AC10 to generate a cytosolic cAMP pool. Cyclic AMP has been known to regulate proliferation, and recently AC10 was observed to be overexpressed in prostate carcinoma, suggesting a role for AC10 in cellular proliferation. Further, pH is also known to regulate proliferation; however, the independent role of bicarbonate in proliferation has not been investigated. Thus, we tested the hypothesis that bicarbonate is required for proliferation of pulmonary endothelial cells.

METHODS: Pulmonary microvascular endothelial cells (PMVECs) and pulmonary artery endothelial cells (PAECs) were seeded at 100,000 cells per well in media with and without bicarbonate. The pH was adjusted similar values and maintained by adjusting incubator CO2 levels. The cells were counted for seven consecutive days to generate growth curves and blood gas analysis was performed to determine pH, pO2, pCO2, and HCO3- concentrations of the media. Additionally, a separate set of PMVECs were seeded at 100,000 cells per well in bicarbonate free media and bicarbonate was added back to the media after 24, 48, 72, 96, 120, 144, and 168 hours and counted five days following the bicarbonate addition.

RESULTS: The growth curves generated by the seven-day cell counts revealed that PMVECs and PAECs seeded in the media with bicarbonate reached log phase growth and eventually a plateau phase; however, PMVECs and PAECs seeded in the bicarbonate free media did not enter log phase growth. When bicarbonate was added back to PMVECs initially grown in a bicarbonate free environment, the cells proliferated to numbers comparable to PMVECs seeded in bicarbonate containing media throughout the growth curve; however, if PMVECs were incubated in bicarbonate free media for more than 96 hours, then these cells did not reach numbers similar to cells seeded in media with bicarbonate.

CONCLUSION: Therefore, the presence of bicarbonate alone allowed cells to proliferate. These finding suggest that bicarbonate is essential for cell proliferation in rat PMVECs. The mechanism by which bicarbonate is utilized for cell proliferation is unknown. Thus, studies regarding whether bicarbonate stimulates AC10 or serves as a carbon source should be considered.
ABSTRACT

DETERMINATION OF THE ENDOCYTIC MECHANISMS OF MICROPARTICLE UPTAKE IN PULMONARY ENDOTHELIUM. Michael Marfice. Sponsored by Natalie Bauer, Ph.D., Department of Pharmacology, Center for Lung Biology, University of South Alabama College of Medicine, Mobile, AL.

Microparticles (MPs) are membrane bound vesicles containing biological matter such as nucleic acids, proteins, and secondary messengers. MPs released by cells, constitutively and upon stimulation, contribute to a diversity of physiological processes such as intercellular signaling, waste management, and coagulation. Previous work in our lab has shown that MPs interact with early endosomes and the trans golgi network following endocytosis by pulmonary vascular endothelial cells (PMVECs), though the specific mechanism by which MPs are endocytosed by PMVECs is undetermined. Caveolin (CavME) and Clathrin mediated endocytosis (CME) are two distinct and well characterized mechanisms of endocytosis; therefore, both were examined as potential facilitators of MP entry. MPs were collected from the media of PMVECs, isolated by serial centrifugation, and labelled with PKH67, a fluorescent membrane label. Naïve PMVECs were pretreated with Filipin or MDC, inhibitors of CavME and CME respectively, and subsequently treated with the labelled MPs for an allocated treatment time. Following treatment, PMVECs were fixed and labelled with Rab5 or TGN38, fluorescent antibodies for the early endosome and the trans golgi network respectively, and DAPI to identify the nucleus to determine the intracellular distribution of MPs. Treated PMVECs were visualized by confocal microscopy to generate images that were analyzed by CellProfiler to calculate the number of MPs within PMVECs. Our results suggest CavME has a negligible, if any, role in active MP uptake, and the likely endocytic mechanism is CME-dependent.
ABSTRACT

ASSESSING THE IMPACT OF NEOINTIMAL LESIONS ON PULMONARY VASCULAR COMPLIANCE AND RESISTANCE. Jorden Smith. Sponsored by Chun Zhou, M.D., Ph.D., Michael Francis, Ph.D, and Troy Stevens, Ph.D., Department of Physiology and Cell Biology, Center for Lung Biology, University of South Alabama College of Medicine, Mobile, AL.

Rationale: Pulmonary arterial hypertension (PAH) is defined as a chronic increase in pulmonary blood pressure (> 25 mm Hg). Medial hypertrophy, medial hyperplasia, and adventitial thickening occur in early stages of the disease process. As the disease progresses, neointimal occlusive lesions develop within pulmonary arterioles, leading to narrowing of vessel lumens and eventually vascular occlusion. It was originally thought that these lesions are relatively uncommon, and do not contribute significantly to the increase in pulmonary artery pressure. However, recent three dimensional reconstruction of hypertensive arterioles suggests that lesion density is more widespread than previously thought. At present, physiological approaches do not discriminate whether or not occlusive lesions impact pulmonary artery pressure. Here, we test the hypothesis that retrograde perfusion will enable quantitative assessment of the impact of occlusive lesions on pulmonary vascular pressures.

Methods: In our experiments, PAH was induced in male Fischer rats via a single injection of SUGEN 5416 (20 mg/kg) followed by exposure to hypoxia (10% O₂). These animals were studied after one (1 Week PAH) and three (3 Weeks PAH) weeks in hypoxia, and after three weeks of hypoxia plus two additional weeks in normoxia (5 Weeks PAH). To examine pulmonary vascular resistance and compliance, the heart and lungs were isolated, ventilated, and perfused with 6% whole blood in both forward and retrograde orientations, beginning at 8 mL/min. Thereafter, flow rates were increased by 8 mL/min every 5 minutes until tracheal edema was visible. Pulmonary artery (Ppa) and pulmonary venous (Ppv) pressures were measured continuously via a physiograph recorder, and double occlusion (Pdo) pressures were measured at the end of each 5-minute interval. In this experiment, double occlusion pressure is representative of pulmonary capillary wedge pressure.

Results: In experimental PAH, Ppa and Fulton Index increase and vascular compliance decreases as the disease progresses. PAH lungs accommodated forward perfusion over the entire 5-week time course. However, only 1 and 3 week PAH lungs accommodated retrograde flow. Retrograde flow was unsuccessful in 5 week PAH lungs, at the time point when occlusive lesions became prominent.

Conclusion: Experimental PAH is a progressive disease that is due to medial and adventitial remodeling and occlusive lesion formation. These occlusive lesions appear to prevent retrograde perfusion, suggesting they are more prominent than previously thought.
ABSTRACT

COLLEGE STUDENT OBESITY PREVENTION PROGRAM: PILOT STUDY. Christen Carter. Sponsored by Sharon Fruh, Ph.D., RN, FNP-BC, Rebecca J. Graves, Ph.D., Heather Hall, Ph.D., Debra Swanzy, DNP, Theresa Wright, DNP, College of Nursing, University of South Alabama, Mobile, AL.

Background: Obesity is becoming an epidemic in the United States; over two-thirds of adults are overweight or obese. The proportion of overweight adolescents has had a three-fold increase in the past 30 years. Young adults 18-29 years of age, with some college education have the greatest increase in the prevalence of overweight and obesity. College students may be more at risk in Alabama since adults in this state have the second highest rate of obesity in the United States. College students are at risk for weight gain once enrolled into college. After graduation, college students are more susceptible to fall into sedentary behaviors because of the stress of starting a career and a family. Early adulthood weight gain increases the risk of chronic diseases in the middle-age. Research has shown that intervention and prevention programs can be effective.

Specific Aim: To identify specific information of nursing students related to: a) height and weight, b) healthy eating habits, c) specific dietary intake habits over the past 30 days, d) perceptions related to healthy food intake, e) confidence level related to healthy eating, and f) physical activity levels using the BUQS: Live Well electronic survey.

Results: Twenty-two nursing students between the ages of 18-33 responded to the survey. The self-reported mean BMI was 24. Fifty percent indicated that they are neutral or dissatisfied with their energy levels and feelings of healthiness. Forty-one percent indicated that they were neutral or dissatisfied with their personal appearance. Currently, 45.5% are trying to lose weight. When asked about their ability to eat less, 45.5% identified that it was somewhat or very difficult to eat less daily. Avoidance of foods high in salt was identified by 59.1%, and 50% avoided foods high in fat. Servings of fruits and vegetables were two or less per day by 77.3% of respondents. Fifty-nine percent indicated that they were too busy to exercise, and 68.2% did not use a pedometer.

Conclusion: The participants are at increased risk of developing obesity based on the mean BMI, difficulty changing eating habits, and barriers to exercise. A support system could be developed which would include faculty and students to promote a healthy lifestyle. This support system would focus on healthy eating, weight loss, and increasing exercise. Another focus of the support system could be to increase servings of fruits and vegetables each day. Based on the findings, researchers plan to conduct a study with a larger sample of college students from multiple colleges across the university campus.
ABSTRACT

HEALTH POLICY AND CHILD POVERTY: AN ILLUSTRATION OF THE SOCIAL DETERMINANTS OF HEALTH.  Aryne Hudson.  Sponsored by Dr. Errol Crook, MD. and Shannon M. Shelley-Tremblay, JD, University of South Alabama, Center for Healthy Communities, Center of Excellence Mobile, AL.

Social determinants of health are conditions in which people are born, live, learn, work, and age that affect a wide range of health, functioning, and quality of life outcomes and risks. Determinants such as social environment, economic environment, income, genetics, transportation, education and more all help determine the health of individuals. Poverty is a socioeconomic condition that has adverse effects on the health of individuals. Access to education, transportation, safe housing, and other social determinants are effected by poverty. The purpose of this study was to identify the effects of poverty on children’s health and the local, state, and national health policies in place to counteract such effects. This study was conducted through a literature review framed around a series of questions targeting the relationship between childhood poverty, the social determinants of health, and health policy. This series includes: What is health equity and how is it different from equality? What is health policy and how do social determinants of health and health policy interact? And what are the consequences of childhood poverty on health? Housing, education, and transportation, are social determinants of health that are strongly influenced by poverty, are often interrelated, and have rippling effects on childhood health. Children living in poverty experience worse health outcomes that impact them throughout their adult lives. For example, poor quality housing can increase health risks such as respiratory and cardiovascular diseases. The social environment of neighborhoods can impact access to education and healthcare. Health policies implemented to prevent or counteract the effects of poverty on childhood health include programs like Medicaid, Children’s Health Insurance Program (CHIP), the Supplemental Nutrition Program for Women, Children, and Infants (WIC) and more. Health policy initiatives that are designed to impact the effort of childhood poverty on children’s health are important to achieve health equity.
ACCESS TO EMPLOYER PROVIDED HEALTH INSURANCE IN LOW INCOME COMMUNITIES. Jasmine Mabry. Sponsored by Kenneth Hudson, Ph.D., Department of Sociology, Anthropology, and Social Work, University of South Alabama, and Errol Crook, M.D., Abraham A. Mitchell Professor and Chair, Department of Medicine, University of South Alabama College of Medicine, Mobile, AL.

Data from the Annual Social and Economic Survey indicates that the majority of adult Americans with health insurance obtain their coverage through their employer or union. Prior research, however, suggests that individuals in low income communities are less likely to have jobs that provide them with health insurance than people who live in more affluent neighborhoods. In this study we use data from the Labor Market Health Care Survey, 2006-2017 (N=231), to examine the likelihood that individuals in high poverty census tracts in Mobile County, Alabama will obtain a job that provides them with health insurance. We use survival analysis to estimate the proportion of this population that will transition during their life-course into a job with employer provided health insurance. Although we expect that most Americans who transition to a job with health insurance will continue to have a job or jobs that provide this benefit until they retire, we examine whether or not this is true for the individuals in our study. The data used in this study was collected using a two-stage probability sample. The low income population in this study is defined by census tracts where 50% or more of the residents have family incomes below the federal poverty threshold. In these communities there is a strong correlation between percent poor and percent black ($\rho = .774, p < .000$). Consequently, about 98% of our randomly selected participants are African American. RESULTS: First, our examination of the survival functions shows that approximately 37% of adults in this population never obtain a job with employer provided health insurance. Although there is not a significant sex difference in the survival functions, those individuals who do not finish high school are much less likely than others to ever obtain a job that provides health care coverage (the modal category of educational attainment is high school diploma). We also find that 63% of adults who do transition into a job with health insurance are not able to sustain employment that provides health care coverage until they reach the minimum retirement age at 62.
Inflammation has emerged as a contributor in the progression of cardiovascular diseases and associated risk factors. We have recently identified in a health care vulnerable population in Mobile County (Alabama) that inflammation is an independent risk factor for the development of diabetes, a major health threat leading to cardiovascular disease. Importantly, our observations were made assessing elevated C-reactive protein as an index of inflammation, a sensitive but unspecific biomarker to diabetes or cardiovascular diseases in general. Caspase-1 is a cysteine protease that cleaves IL-1β and IL-18 into their active inflammatory forms shown to reduce insulin sensitivity in mice, and thus contributing to the development of diabetes. However, whether caspase-1 is elevated in individuals with diabetes or associated with the severity of the disease is unknown. Therefore, we sought to determine the levels of caspase-1 from a diabetic population in Mobile County, a region identified as a part of the diabetes belt in the United States. To address our goal we obtained Institutional Review Board approval to analyze plasma samples collected from diabetic subjects residing in health disparity communities in Mobile County. Plasma samples from 13 individuals identified as diabetics with various levels of elevated glycosylated hemoglobin (HbA1c) were assessed by an ELISA assay following manufacturer recommendations. Samples were measured in triplicates and corresponded to two individual clinical visits separated in a range of 3 to 12 months. Average levels of triplicates are reported. Levels of caspase-1 were significantly elevated compared to a control cohort of 7 individuals with no history of diabetes (147 vs. 68 pg/mL, respectively, unpaired t-test). No differences were identified in samples analyzed from the two time points, a result that was consistent with the absence of differences in HbA1c at the same time points. The data indicate that from a small cohort of patients with diabetes in Mobile County, caspase-1 is elevated compared to subjects with no diabetes but does not correlate with the severity of the disease. We conclude that caspase-1, a specific biomarker of inflammation that associates with the progression of cardiovascular diseases, constitutes a novel biomarker of diabetes.
ABSTRACT

TELEHEALTH ENHANCED EDUCATION FOR PATIENTS LIVING WITH DIABETES IN RURAL ALABAMA. Elizabeth Torrance\textsuperscript{1}. Sponsored by Jessica Hardy, MPH, DNP, APRN, ACNS-BC\textsuperscript{2} and Alethea N. Hill, Ph.D., ACNP-BC, ANP-BC\textsuperscript{3}University of South Alabama CHC Program, Mobile, AL  \textsuperscript{2}Alabama Public Health Department, Montgomery, AL  \textsuperscript{3}University of South College of Nursing, Mobile, AL

BACKGROUND: Recent studies show that Alabama is ranked 46th for overall health and diabetes. According to the CDC, diabetes is a prevalent cause of death in America. In 2013, 14 percent\textsuperscript{4}, approximately 510,000 Alabamians were diagnosed with diabetes. This study was designed to take place in Escambia County, to observe the effective or defective results on educating patients diagnosed with diabetes by utilizing telehealth education.

PURPOSE: The purpose of this project, is to provide a unique opportunity to provide diabetes education and prevention, and by doing so, have a positive impact on the patient’s A1c levels.

OUTCOMES: The primary outcome is to observe diabetic self-management, self-care, and self-efficiency changes at 12 weeks post the intervention. Secondary outcome is to observe the participants' hemoglobin A1c, plasma glucose, blood pressure, waist circumference, BMI, lipid panels, and physical fitness; and how it directly correlates to this intervention. Outcome Measures and Benchmarks: (1) Increase in the number of individuals in Escambia county who are educated on diabetes management and prevention; (2) Increase in knowledge of telehealth technology use for diabetes education; (3) Increase in the number of diabetes management and prevention messages that are disseminated throughout Escambia county.

METHODS: The recruitment for this convenience sample was identified by referral, local health departments, advertising and self-reported history of diabetes. Pre and post test assessments will be performed for primary and secondary outcome measures. The Summary of Diabetes Self-Care Activities scale (SDSCA) will measure the seven self-care behaviors of DSME. The scale includes a self-report of the number of days in the week the subject engaged in the following activities: a) eating a generally healthy diet, b) eating a diet high in fruits/vegetables and low in fat, c) physical activity lasting greater than 30 minutes, d) glucose self-monitoring, e) foot inspection, and f) taking medication. The Problem Areas in Diabetes (PAID) scale will assess perception of stress related to diabetes management, specifically, psychological adjustment to diabetes focusing on feelings about the complexity of self-management.

IMPLICATIONS: Use of telehealth technology to enhance evidence-based diabetes self-management curriculum has the potential to reach and empower individuals living with diabetes in rural Alabama with tools to make informed decisions for self-care; engage in effective diabetes self-management; and implement self-care behaviors to experience optimal physical and psychological well-being. Participants are expected to complete the study in two weeks.
ABSTRACT

A Mouse Model To Test The Cytotoxic Immune Response To Intracellular Antigens. Raven Walker. Sponsored by Victor Solodushko, Ph.D. and Brian Fouty, MD. Departments of Pharmacology and Internal Medicine. University of South Alabama College of Medicine, Mobile, AL

Intracellular organisms cause a number of important human diseases such as malaria, tuberculosis, and melioidosis. The lack of a reliable animal model to study the cytotoxic response against such organisms has hindered the development of vaccines to fight these diseases. While these organisms may express many antigens, only a few are likely to be sufficiently immunogenic to induce a strong cytotoxic immune response. The goal of this project was to develop a mouse model to test the in vivo cytotoxic response to different intracellular antigens. CT26WT cells are a colon carcinoma cell line that causes tumors when injected into immunocompetent BALB/c mice. CT26WT cells were engineered to express the weak non-bacterial antigens green fluorescent protein (GFP) or mCherry. Other CT26WT cells were engineered to stably express two proteins (TssM and Hcp1) which are known to be strong antigens partially responsible for the cytotoxic immune response against cells infected with *B. pseudomallei*, the intracellular gram-negative bacteria that cause melioidosis. These engineered CT26WT cells were also tagged with mCherry so tumor growth could be assessed over time. Cells were injected subcutaneously into mouse-tails and fluorescent expression determined over 5 weeks. One group of mice were injected with an equal number of GFP-expressing and TssM/Hcp1/mCherry-expressing cells while another group of mice were injected with an equal number of GFP-expressing and mCherry-expressing cells (i.e. mCherry with no antigen attached). Mice that were injected with CT26WT cells that expressed GFP or mCherry remained invisible to the immune system and generated progressively larger tumors that expressed both GFP and mCherry. Mice injected with CT26WT cells that expressed GFP or Hcp1/TssM/mCherry formed a tumor during the first 2 weeks that expressed both GFP and mCherry equally. Between 2 and 3 weeks, however, the Hcp1/TssM/mCherry-expressing cells were rapidly eliminated, leaving only GFP-positive cells in the tumor. This suggested that a cytotoxic immune response had been activated against the Hcp1/TssM/mCherry cells due to the presence of the (strong) *B. pseudomallei* antigens, but a cytotoxic immune response was not generated against the (weak) antigens, GFP or mCherry. By discriminating between strong and weak immunogenic antigens, this proposed method can be used to study the cytotoxic immune response to other known or unknown intracellular antigens of interest, and thus, guide the development of novel DNA vaccines against intracellular organisms.

[Funding for this program was made possible by 1P20MD0002314 from the National Center on Minority Health and Health Disparities]
ABSTRACT

Pseudomonas aeruginosa pneumonia induces lung production of cytotoxic amyloids, which contribute to adverse outcomes in critically ill patients. Acidosis is common among these patients, and is also associated with poor outcomes. Pulmonary capillaries are exposed to acidosis during infection, yet it remains unclear how these cells sense and respond to acidosis. Carbonic anhydrase IX (CA IX) converts proton to carbon dioxide, alleviating cellular acidic stress. We recently found that pulmonary microvascular endothelial cells (PMVECs) express high levels of CA IX. Thus, we hypothesized that CA IX reduces PMVEC acid exposure, and in so doing, protects the cells from amyloid-induced cytotoxicity. PMVECs were isolated from Sprague Dawley rats. Cytotoxic amyloid supernatant was generated by treating PMVECs with PA103 for 4 hours and filter sterilizing the supernatant. Naïve PMVECs were treated with cytotoxic amyloid supernatant over a pH range (7.4 to 6.2), in the presence or absence of the CA IX inhibitor, SLC-0111, for 6 hours. Cells were photographed and counted. Supernatant was collected for pH measurements and lactate dehydrogenase (LDH) cytotoxicity assay. After supernatant collection, cells were incubated with regular media with pH 7.4 for 3 days for recovery. Cytotoxic amyloid supernatant induced acidosis in PMVECs (control vs. amyloid, pH 6.9 vs 6.6, P <0.05). Acidosis enhanced amyloid cytotoxicity in a dose dependent manner assessed by microscopic exam and LDH release (pH 7.4 vs. 6.8 vs. 6.2, 1.0 vs. 12.0 vs. 36.4% of max value, P <0.05). In the presence of SLC-0111, the enhancing effect of acidosis on amyloid cytotoxicity was further accelerated, suggesting that CA IX plays an important role in PMVECs survival. During recovery phase, when cytotoxic amyloid supernatant removed, pH normalized and the cells that were exposed to acidosis during amyloid challenge displayed improved survival; at pH of 7.4, 6.8, and 6.2 cell counts were 2.4, 3.3, and 6.3 x 10^5 cells, respectively (P <0.05). SLC-0111 pre-treatment abolished the protective effect of acidosis during recovery, suggesting CA IX is an important mediator of PMVECs survival during both injury and repair phases of lung injury. CA IX is an important determinant of PMVECs survival during injury and repair phases of lung injury.
ABSTRACT

PULMONARY ARTERIAL HYPERTENSION VIA SUGEN/HYPOXIA IMPAIRS PULMONARY ARTERY ENDOTHELIAL CELL PROLIFERATION, AND ALTERS CELL MORPHOLOGY. Bradley Schuler. Sponsored by Michael Francis, Ph.D., Department of Physiology and Cell Biology, Center for Lung Biology, University of South Alabama College of Medicine, Mobile, AL.

Pulmonary arterial hypertension (PAH), defined as elevated pulmonary artery pressure (>25 mmHg), is driven by vasoconstriction and vascular remodeling that leads to the formation of occlusive lesions within pulmonary arterioles. Occlusive vascular remodeling in PAH is irreversible, and is caused in part by the abnormal growth and morphology of endothelial cells. Therefore, we hypothesized that in PAH, there is greater proliferation of pulmonary arterial endothelial cells (PAECs). Furthermore, increased expression of Transient Receptor Potential (TRP) channels has been implicated as a cause of disorganized angiogenesis and vascular cell proliferation in PAH. Given that our previous studies have shown that the canonical TRP protein, TRPC4, is associated with increased endothelial dysfunction and lesion severity in PAH, we hypothesized that endothelial TRPC4 expression would be upregulated in PAH. To test our hypothesis, we studied PAECs derived from the Sugen/hypoxia (Su/Hx) rat model of PAH. We compared the proliferative characteristics and morphology of endothelial cells from both control and Su/Hx Fischer rats by performing growth curves, microscopy, and image analysis. In contrast with our expectations, growth curve analysis showed that Su/Hx PAECs cells actually grew at a rate comparable to control (mean doubling time was ~1.5 days for both groups). However, Su/Hx cells reached a lower plateau (262k ± 23k cells vs 605k ± 27k cells in control), and image analysis revealed a more “plump” cell morphology characterized by an increased cell diameter. Using immunofluorescence microscopy, we measured the localization and relative amount of TRPC4 expression. In control cells, TRPC4 was only expressed at the cell periphery, and was uniformly distributed throughout the cell population. In contrast, TRPC4 was expressed abundantly and diffusely throughout Su/Hx cells, but only in a subset of the total cell population. Although it is clear that PAH induces the abnormal growth of pulmonary endothelial cells, our results indicate that this is not simply due to an increase in cell number. Additionally, heterogeneously upregulated TRPC4 may drive abnormal endothelial cell morphology and function in PAH, and underlie vascular lesion progression. We next plan to evaluate these responses in rats genetically lacking TRPC4, to determine whether blocking this specific protein could ultimately derail vascular remodeling before it begins.
CHEMICAL PROPERTIES OF ENDOTHELIAL CYTOTOXIC AMYLOIDS. Reece Stevens, Eugene Cioffi, Sarah Voth, and Ron Balczon. Sponsored by Ron Balczon, Ph.D., Department of Biochemistry & Molecular Biology, Center for Lung Biology, University of South Alabama College of Medicine, Mobile, AL.

*Pseudomonas aeruginosa* infection induces endothelial production of cytotoxic amyloids, such as oligomeric tau and beta amyloid. 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) has been used by various investigations to disrupt amyloids. In this study, the susceptibility of endothelial- produced amyloids to HFIP was investigated. In initial studies, supernatant obtained from *P. aeruginosa*-infected rat pulmonary microvascular endothelial cells (PMVECs) was treated with concentrations of HFIP ranging from a 10:1 ratio to a 0.25:1 ratio, and then levels of intact amyloids were measured spectrophotometrically using Thioflavin T (ThT). ThT fluorescence decreased with increasing HFIP concentrations. Cytotoxicity was then assessed by adding HFIP treated supernatants to cultured PMVECs, and cytotoxicity was abolished at all concentrations of HFIP used. These results demonstrate that cytotoxins produced by PMVECs following *P. aeruginosa* infection are susceptible to dissociation by HFIP, consistent with an amyloid nature. Supported by Center for Lung Biology T32 Training Grant HL076125.
ABSTRACT

DEVELOPMENT OF A FLOW CYTOMETRY-BASED IMMUNOASSAY (FLISA) TO DIAGNOSE PULMONARY ALVEOLAR PROTEINOSIS IN MICE. Hannah Klein. Sponsored by Robert A. Barrington, Ph.D., Department of Microbiology & Immunology, Center for Lung Biology, University of South Alabama College of Medicine, Mobile, AL.

Autoantibodies (AAB) specific for granulocyte colony-stimulating factor (GMCSF) cause autoimmune pulmonary alveolar proteinosis (PAP), a rare restrictive lung disease characterized by accumulation of surfactants in the lower airways. We recently discovered a strain of mouse that also develops autoantibody-dependent PAP (Ferretti et al. 2016 J Immunol 197:470). To date, an ELISA detecting GMCSF-binding AAB serves as the main laboratory test used to diagnose the disease in both human and in mice. However, that AAB are surprisingly detectable in serum from healthy individuals (in addition to patients) has raised the need for an independent assay to validate the specificity of GMCSF-binding AABs. To provide an independent assay to diagnose PAP, we present the development of a flow cytometry-based immunoassay (i.e. FLISA) to detect GMCSF-binding AABs using the mouse model as a proof of concept. The assay utilizes stable transfected human embryonic kidney cells (HEK293) expressing murine GMCSF as the extracellular domain of a fusion with transmembrane amyloid precursor protein (APP). These GMCSF-APP expressing cells thus provide a folded protein rather than a solid phase-bound molecule used for ELISA, providing the potential for more native antibody-antigen interactions.

The plasmid encoding GMCSF-APP contains a neomycin resistance gene as well as the gene for green fluorescence protein (GFP, from Aequorea coerulescens), allowing transfected HEK293 cells to be selected for by growth with G418 as well as to be easily identified by green fluorescence. To evaluate the sensitivity of antibody detection, binding studies were performed using biotinylated monoclonal rat anti-mouse GMCSF. Bound antibody was detected using streptavidin-allophycocyanin (APC), and mean fluorescence intensity of APC (MFI) was quantified. Corresponding MFI values using 625, 312.5, 156, 78, 39, 19.5, 10 and 5 ng/ml antibody showed a linear correlation between antibody concentration and MFI. In addition, the MFI of GMCSF-APP expressing HEK293 cells receiving no antibody (streptavidin-APC only control) was comparable to the MFI using 10 ng/ml antibody, indicating that the FLISA could detect approximately 10-20 ng/ml bound antibody.

We earlier established, by ELISA, that serum levels of anti-GMCSF AAB in mice developing PAP ranged from 20-84 ng/ml, while levels in control mice without disease ranged from 2-20 ng/ml. Future experiments will seek to improve FLISA sensitivity and also to test detection of GMCSF AAB from mouse sera.
Inflammasomes are germ-line encoded pattern recognition receptor complexes that assemble in response to danger signals elicited by environmental stresses such as infection and sepsis. Inflammasome assembly elicits auto-activation of Caspase-1, a cysteine dependent active site, aspartate specific protease that coordinates host cell stress responses. While classically studied in innate immune cells, recent findings from our laboratory indicate that Caspase-1 is activated in endothelial cells as part of a novel innate immune response to infection with the opportunistic Gram-negative pathogen, *Pseudomonas aeruginosa*. The present study is based on our observation that *P. aeruginosa* infection stimulates glucose metabolism in cultured pulmonary microvascular endothelial cells (PMVECs) beyond that of either the host or pathogen alone. Considering that Caspase-1 has recently emerged as a negative regulator of glycolytic enzyme function, we tested whether culture medium glucose concentrations affect Caspase-1 activation in response to *P. aeruginosa* infection. To this end, cultured PMVEC monolayers were conditioned for 1 hour in serum-free DMEM containing 22 mM, 5.5 mM, or 0 mM glucose. PMVECs were then inoculated with sterile saline (control) or saline containing 40 *P. aeruginosa* per host cell (multiplicity of infection =40:1). At the time of inoculation PMVECs were loaded with a Fluorescently Labeled Indicator of Caspase-1 Activation (FLICA), which binds irreversibly to active Caspase-1. At 3 hours post infection, cells were harvested and analyzed by flow cytometry. Intriguingly, lowering medium glucose concentration increased the number of PMVECs with activated Caspase-1. Importantly, in the absence of infection lowering glucose concentrations did not activate Caspase-1. In this model, increased FLICA signal correlates with increased PMVEC cell death. Thus, we next measured LDH release to the culture medium as an indication of cell death. Lowering medium glucose concentrations increased the amount of LDH release to the medium. Lastly, time-lapse video microscopy revealed that lowering medium glucose concentrations resulted in morphological differences in the PMVEC response to infection. Together the data suggest that medium glucose concentration and infection-induced changes in host cell glycolytic metabolic flux may represent novel environmental conditions that regulate the PMVEC Inflammasome-Caspase-1 axis responds to *P. aeruginosa* infection. Future studies will be aimed at elucidating the mechanism by which the Inflammasome-Caspase-1 axis senses changing host cell nutrient flux.
ABSTRACT

MITOCHONDRIAL DNA REPAIR: A PHARMACOLOGICAL TARGET TO INCREASE THE POOL OF DONOR LUNGS AVAILABLE FOR TRANSPLANT.
Mariah Stewart. Millsaps College. Jackson, MS. Sponsored by Mark Gillespie, Ph.D., Department of Pharmacology, Center for Lung Biology, University of South Alabama College of Medicine, Mobile, AL.

Lungs donated after circulatory determination of death (DCDD) are underutilized for transplant in part because post-mortem metabolic degradation renders them prone to ischemia-reperfusion (IR) injury. A means to improve viability of DCDD lungs is ex vivo lung perfusion after procurement, but the effectiveness of this restorative strategy is also limited by the propensity for IR injury. Reactive oxygen species generated during lung IR injury oxidatively damage the mitochondrial (mt) genome, causing cytotoxicity and pulmonary vascular endothelial barrier dysfunction. Here we used a fusion protein targeting the DNA repair enzyme, OGG1, to mitochondria to determine if protection from mtDNA damage blunts endothelial dysfunction associated with IR injury in a rat model of DCDD lung procurement. After a 1h post-mortem period, rat lungs were excised, subjected to cold ischemia at 4°C for 2 h, and then mounted in a constant flow perfusion apparatus to mimic protocols used in human lung transplant. In one group, mitochondrial-targeted OGG1 fusion protein (10µg/mL) was added to the perfusion medium and vascular filtration coefficient (Kf) was recorded over a 2h period. After perfusion, the wet:dry lung weight ratio was calculated as an index of edema formation. The extent of mt DNA damage and abundance of mtDNA damage associated molecular patterns (DAMPs) was evaluated in lung tissue and perfusate by quantitative Southern blot and quantitative PCR of selected mitochondrial DNA fragments, respectively. The Kf and wet:dry lung weight ratio were elevated in 1h post-mortem group compared to the no post-mortem group. In addition, mtDNA damage and mtDNA DAMP release were present after the 1h post-mortem and ex vivo perfusion periods as evidenced by a 30% increase in lung tissue mtDNA base oxidation and a three-fold increase of extracellular mtDNA fragments in the perfusate. Treatment with OGG1 prevented the rise in Kf and increase in wet:dry weight ratio that occurred during 1h ex vivo perfusion of post mortem-processed lungs. In addition, OGG1 decreased lung tissue mtDNA damage and accumulation of mtDNA DAMPs in lung perfusate. These findings indicate that cold ischemia is accompanied by mtDNA damage and DAMP release, and that a postmortem delay in lung procurement exacerbates the severity of endothelial barrier dysfunction. Because the fusion protein targeting OGG1 to mitochondria attenuated endothelial barrier degradation, mtDNA damage, and DAMP release in cold stored and DCDD + cold stored lungs, these results demonstrate that oxidative mtDNA damage contributes to vascular injury in DCDD lungs and point to a pharmacologic strategy for enhancing physiologic function in this setting.